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Studies on Neurosecretion in the Alfalfa Plant Bug, *Adelphocoris lineolatus* (Goeze) (Hemiptera : Miridae)

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ABSTRACT

The gross morphology of the central nervous system and the histology of the neurosecretory systems and retrocerebral glands in all stages of postembryonic development of Adelphocoris lineolatus (Hemiptera : Miridae) are described and figured. A new technique for staining neurosecretory products in insects is outlined.

The suboesophageal and first thoracic ganglia are separate, while the second and third thoracic are fused with the abdominal ganglia. Innervation of the female reproductive organs is described. The retrocerebral complex consists of a hypocerebral ganglion, paired corpora paracardiaca, a single corpus allatum, and their associated nerves.

Two types of neurosecretory cells, based on size and on selective stainability, were seen. The most conspicuous type, the Type A cells, are found only in the pars intercerebralis medialis of the protocerebrum. The Type B cells are found in the pars intercerebralis medialis and lateralis, the prothoracic ganglion, and the thoracoabdominal ganglionic centre. The neurosecretory cells, particularily the A cells, both individually and as groups, show periods of activity that can be correlated with stages of development of the insect. The Type A cells apparently trigger the cycle of secretion of the entire endocrine system. The Type B cells of the prothoracic ganglion show much greater activity during the nymphal instars than in the adult stages. A marked increase in the secretory

activity of the A cells during the pre-oviposition period suggests that these cells, along with the corpus allatum, which is also hyperactive at this time, play a role in reproductive physiology. An increase in secretory activity of the B cells of the last ganglion during the latter part of the pre-oviposition period suggests that these cells, too, may function in some way in reproductive physiology.

The postero-ventral part of the corpora paracardiaca contains secretory cells. Neurosecretory colloid from the brain can be traced all along the nervi corporis paracardiaci into this gland, and along with the secretion produced by the cells of the corpora paracardiaca, is probably released into the aorta. This gland serves as a storage organ for neurosecretory material from the brain, and it probably produces its own hormone.

Cycles of activity in the secretory cells of the corpus allatum can be seen in each nymphal instar. The corpus allatum apparently stops secreting about half way through the intermoult period in the fifth (last) instar. In the adult female, the corpus allatum is hyperactive until just before oviposition.

The thoracic glands are located in the mesothorax and are innervated from the suboesophageal ganglion. Cycles of activity can be seen in this gland in each nymphal instar. In the adult, the thoracic glands break down soon after emergence, perhaps due to hyperactivity of the corpus allatum.

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INTRODUCTION

The purpose of this study has been to locate and describe the neurosecretory cells in the central nervous system, and to describe the gross morphology of the central nervous system and the retrocerebral endocrine organs, in all stages of postembryonic development of the alfalfa plant bug, Adelphocoris lineolatus (Goeze) (Hemiptera:Miridae), and, as far as possible, to relate the activity of these structures to moulting, reproduction, and diapause. This investigation has arisen out of a general study on diapause and reproductive physiology of insects, with special reference to those affecting forage crop or forage crop seed production in the northern agricultural areas of Saskatchewan.

Neurosecretory cells differ in their location among the various groups of insects, and the mechanism by which these structures influence reproductive physiology and diapause has not yet been well established. A. lineolatus is an interesting subject from the point of view of studying reproduction and diapause, since the population in northern Saskatchewan, which presumably originated from a bivoltine population in Minnesota, is adapting, or has just adapted, to a univoltine life history[1].

1. Unpublished field notes, Forage Crop Insects Project, Canada Agriculture Research Station, Saskatoon, Sask.

The present study, describing the histophysiology of the neurosecretory cells in all stages of postembryonic development, is the first and perhaps the most important part of this project.

HISTORICAL

A number of excellent reviews of the literature on endocrine control in insects have appeared during the past ten years (Bodenstein, 1954, 1957; Gabe, 1954a; Karlson, 1956; Novák, 1959; Pflugfelder, 1952; Scharrer, 1952a, 1953, 1954, 1955a, 1957; Scharrer and Scharrer, 1954; Wigglesworth, 1954a). Only the more significant advances in the field will be discussed here.

According to Cazal (1948) the first descriptions of endocrine organs in insects were of the thoracic glands (= prothoracic glands)[2] by Lyonet (1792), and of the corpora allata by Müller (1828) and Straus-Durckheim (1828). Endocrine functions were first ascribed to the thoracic glands by Toyama (1902), and to the corpora allata by Nabert (1913). De Lerma (1933) was the first to show that the corpora paracardiaca (= corpora cardiaca)[3] consisted of glandular as well as nervous elements.

Kopeč (1922) was the first to suggest that the moulting process in insects was controlled by hormones. He showed that the brain was acting as an endocrine organ in Lymantria (= Porthetria) dispar; removal of the brain prevented moulting while re-implantation of the brain

2. See p. 65.

3. I believe the term "corpus paracardiacum" for these bodies is more appropriate, since their connexion with the heart is entirely secondary (Snodgrass, 1954; Ewen, 1960).

promoted further moulting and metamorphosis. Wigglesworth (1934, 1936) then showed that there was a critical period necessary for the release of the moulting hormone in Rhodnius prolixus. If the Rhodnius nymph was decapitated before the critical period had been reached, no moulting would occur; but if the operation was carried out after the critical period had been reached, moulting was not prevented. Wigglesworth first suggested that the moulting hormone was produced by the corpus allatum, but he modified his original hypothesis after the description of the medial neurosecretory cells in the brain of Rhodnius by Hanström (1938), and proved that moulting was triggered by a hormone secreted by these neurosecretory cells (Wigglesworth, 1940). By transplanting the medial neurosecretory cells from donor insects that had reached the critical period into nymphs that had been decapitated before the critical period, Wigglesworth proved that these cells would induce moulting. This role of the brain in the moulting process has been demonstrated in many other insects (e.g., Bodenstein, 1954; Scharrer, 1952a, 1953; van der Kloot, 1960).

The first suggestion that the brain did not act alone in the control of moulting was given by Hachlow in 1931. By ligaturing Vanessa sp. larvae at various levels, he found that a thoracic growth centre was also involved. Plagge (1938) demonstrated that implanted

brains alone could not induce isolated abdomens of Deilephila euphorbiae larvae to moult. Following up these results, Fukuda (1940a,b) proved that the thoracic glands produced a hormone that took part in the moulting cycle of Bombyx mori. Piepho in 1942 and later Williams (1947a,b, 1949), suggested that the brain and thoracic glands functioned as an endocrine system, with the former stimulating the latter to produce and secrete their hormone, and that it was the hormone of the thoracic glands that acted on the tissues of the body at moulting. This thoracotrophic action of the brain hormone has now been demonstrated in many insects. The information available indicates that the brain hormone is blood-borne and that a nervous connexion between the brain and the thoracic glands, or their homologues, is not necessary for its trophic action. However, Possompès (1953) demonstrated that these nervous connexions must be intact to enable the thoracic glands of Calliphora erythrocephala to be activated. As yet this has not been found to be so in other insects, but the possibility of a nervous stimulation should not be overlooked since neurosecretory cells are capable of transmitting nervous impulses, at least in some animals (Potter and Loewenstein, 1955; Carlisle, 1957). Butenandt and Karlson (1954) were the first to isolate an insect hormone in pure form. Using silkworm pupae, they isolated the hormone of the thoracic gland,

which they called ecdysone. Subsequently it was shown that ecdysone is either a protein or attached to one (Schmidt and Williams, 1953), and Wigglesworth (1957) has suggested that it probably acts by affecting permeability at cell surfaces.

The thoracic gland degenerates in most insects at the time of metamorphosis. This disappearance ensures that the adult insect shall not moult again. In Ephestia kühniella (Rehm, 1951) and Drosophila virilis (Bodenstein, 1947), the gland breaks down in the late pupal stage. In Calliphora (E. Thomsen, 1943), Odonata (Pflugfelder, 1947), Rhodnius (Wigglesworth, 1952a), and Periplaneta americana (Bodenstein, 1953) degeneration begins after the imaginal moult. In Thysanura, which moult throughout their adult life, the thoracic glands, or "ventral glands", persist (Gabe, 1953a).

Whether or not the moult, which is initiated by the action of the thoracic gland hormone on the effector organs, results in a larva, pupa, or imago, depends on the action of a third endocrine gland, the corpus allatum. As mentioned previously, the corpus allatum was first suspected of being the source of the moulting hormone (Wigglesworth, 1934). Bounhiol (1938) and Plagge (1938), however, noted that caterpillars from which the corpora allata had been removed could still moult. In 1934 Wigglesworth showed that a precocious

adult could be produced by decapitating a young Rhodnius nymph shortly after the critical period. By joining fourth stage nymphs sectioned at various levels of the head to fifth (last) stage nymphs, he was able to show the corpus allatum to be the source of a hormone that suppressed adult development in the larval insect. He called this hormone the juvenile hormone. His results indicated that the juvenile hormone promoted differentiation of the larva and inhibited that of the imago. Furthermore, it has been shown that by implanting corpora allata from adult donors, last stage larvae can be made to undergo extra larval moults. This was observed in Carausius (= Dixippus) morosus (Pflugfelder, 1940, cited in Wigglesworth, 1954a), Melanoplus differentialis (Pfeiffer, 1945), and Rhodnius (Wigglesworth, 1948). Similar results have been obtained with Galleria mellonella (Piepho, 1942, 1943). If corpora allata from young larvae are transplanted to full-grown larvae, larval development is continued with the eventual production of giant pupae and imagines. Conversely, if the corpora allata are removed from young larvae they give rise to small pupae and imagines. The suppression of adult characters by implanting additional corpora allata and the precocious appearance of adult characters after allatectomy has since been reported in larvae of many insects, including Carausius (= Dixippus) (Pflugfelder,

1937, 1939, 1952), Leucophaea maderae (Scharrer, 1946a), and Apis mellifica (Schaller, 1952). Thus, when the corpus allatum hormone is present and acting simultaneously with the thoracic gland hormone, a larval moult results. However, in the absence of the juvenile hormone, or where there is a decrease in its activity relative to ecdysone, the moult will be regulated by the hormone of the thoracic gland; holometabolous insects moult to pupae, and hemimetabolous insects to imagines.

Metamorphosis, however, does not take place in a single step. Gillett (1935) described a slight differentiation of imaginal characters at each larval moult in Rhodnius, although the changes which occurred at the fifth moult (metamorphosis) were far more extensive. For example, he reported that the rudiments of the external genitalia usually appeared in the third instar and the sexes were quite distinct in the fifth stage.

Varying degrees of metamorphosis can be brought about by decapitation at different times after the critical period; this has been demonstrated in Rhodnius (Wigglesworth, 1934), Bombyx (Fukuda, 1944), and Sialis lutaria (Rahm, 1952). These results indicated that the juvenile hormone was secreted after the moulting hormone of the thoracic gland. Wigglesworth (1936, 1952b) proved this to be so in a series of experiments on Rhodnius. He showed that the time of release and the

concentration of the juvenile hormone were responsible for the degree of metamorphosis at any moult. Schaller (1952) suggested that, from the point of view of hormone action, the larval - pupal transformation of the holometabola is similar to the nymphal - adult change in the hemimetabola. By ligating or decapitating Apis larvae at the appropriate time, Schaller showed that the pupal stage could be omitted and the larvae made to moult to almost complete adults. These results suggest, then, that it is the juvenile hormone that maintains the integrity of the juvenile tissues, and that the final histolysis of the specialized larval tissues in the holometabola is due to a reduction or cessation of activity by the corpus allatum in the last larval instar (Snodgrass, 1954). However, although the juvenile hormone seems to inhibit development in the larval tissues, the imaginal buds of the holometabola continue to grow during the larval instars. For example, Eassa (1953) reported that mitosis was observed in the cells of the antennal discs in larval Pieris brassicae. Thus it seems that the juvenile hormone is selective for larval tissues, and/or that the cells of the imaginal buds are not affected by it (Snodgrass, 1954).

The foregoing results suggest that moulting and metamorphosis are controlled by a delicate hormone balance between the products of the corpus allatum and

the thoracic gland. During the larval stages, the juvenile hormone suppresses the appearance of adult characters, but at metamorphosis its effects are either over-ridden by the thoracic gland hormone which favours imaginal differentiation (Vogt, 1943b), or there is an actual decrease in the amount of juvenile hormone present, due to a decline in activity of the corpus allatum. At any rate, metamorphosis is brought about by some shift in the hormone balance. After metamorphosis, the thoracic gland breaks down and no further moulting can occur. This breakdown of the thoracic gland in the adult pterygote insect is initiated by an apparent change in balance among these glands, the corpora allata, and the corpora paracardiaca (Bodenstein, 1953; Wigglesworth, 1954a). Since the important function of the thoracic gland is to induce moulting in the immature insect, it may well be that the maintenance of this organ during development is also dependant upon a delicate hormone balance between it and the corpora allata and paracardiaca. It is important to remember that the thoracic gland is strictly a "larval" structure which undergoes histolysis in the adult.

It is important to note, too, that these "growth" hormones are only the regulators, and not the determiners, of the type of development. Hereditary factors in the animal are the true determiners of the

course of development (Snodgrass, 1954). This is clearly demonstrated by the fact that the hormones, in most cases, are not species-specific, and in many cases not even genus-specific. Furthermore, the state of the tissues is also important in determining the kind of moult. Vogt (1940) noted that the larval ovaries of Drosophila funebris and D. melanogaster had to grow to a certain size before metamorphosis could occur. By implanting imaginal discs into mature D. melanogaster and D. virilis larvae, Bodenstein (1943) showed that while some differentiated, others did not, and in those that did differentiate, their rate of growth depended on the concentration of the thoracic gland hormone.

Besides controlling the events leading to moulting and metamorphosis, hormones have been implicated in a number of other physiological processes in insects. Perhaps the best known of these is the role of hormones in reproduction. It has been shown, for example, that normal deposition of the yolk in the developing egg is inhibited following removal of the corpora allata. This effect was demonstrated in Melanoplus (Pfeiffer, 1939, 1940), Leucophaea (Scharrer, 1946b), Rhodnius (Wigglesworth, 1936), Oncopeltus fasciatus (Johansson, 1958), dytiscids (Joly, 1945), and various flies (Day, 1943; E. Thomsen, 1940, 1943). Joly (1945) proved that Dytiscus spp., which normally lay eggs in March or April, could be induced

to lay in the winter by implanting several pairs of corpora allata. He also showed that the normal cycle of egg development could sometimes be restored by re-transplanting the corpora allata; if a corpus allatum was implanted directly within the ovary, the oocytes immediately adjacent to the implant were the ones most affected. Conversely, ovariectomy is frequently followed by hypertrophy of the corpora allata. This was seen in Calliphora (E. Thomsen, 1943), Lucilia sericata (Day, 1943), Drosophila spp. (Vogt, 1942), Melanoplus (Pfeiffer, 1940), and Rhodnius (Wigglesworth, 1948), but not in Sarcophaga securifera (Day, 1943). Vogt (1942) also noted that this hypertrophy could be reversed by implanting corpora allata into allatectomized Drosophila spp. Nayar (1957, 1958) reported that cyclic changes, which paralleled the phases of the reproductive cycle, occurred in the cerebral neurosecretory cells in Iphita limbata. When the preoviposition period began, the stainable material in these neurosecretory cells decreased, and at the time of oviposition, the blue-staining material had disappeared from the cells. These results agree with those of Dupont-Raabe (1951, 1954b), who noticed intense staining in the neurosecretory cells in egg-laying phasmids. E. Thomsen (1948) demonstrated that the gonadotrophic effect of the corpus allatum was probably controlled by the brain in Calliphora. Removal of the intercerebralis cells in this

insect had an effect similar to allatectomy; the ovaries failed to fully develop. A similar effect was demonstrated in Leucophaea by Englemann (1957). Johansson (1958), however, reported that removal of the medial neurosecretory cells in young female Oncopeltus did not prevent normal egg development. In Drosophila spp., corpora allata from even first instar larvae restored egg development when implanted into allatectomized adults (Vogt, 1943a; Bodenstein, 1947). These results prompted Pfeiffer (1945) to suggest that the juvenile hormone of the larva and the gonadotrophic hormone of the adult were one and the same. However, Bounhiol (1938) and Fukuda (1944) have reported that silkworm caterpillars that had been allatectomized immediately metamorphosed and the resulting moths produced normal eggs. Perhaps in these caterpillars the neurosecretory cells of the brain or the corpus paracardiacum fulfilled the gonadotrophic function.

At a specific stage in their life history, many insects suddenly cease development and enter a state of dormancy, called diapause. Diapause involves developmental arrest whenever it intervenes during embryogenesis, larval, or pupal life; when it occurs in adult life, maturation of the gametes is inhibited. Physiologically, the mechanism of diapause is still in doubt, although it has been known for many years that

insects suffer an arrest of growth when deprived of the source of the moulting hormone. The resemblance of this condition to the natural state of diapause was first noted by Wigglesworth (1934). He found that if a Rhodnius nymph was decapitated immediately after the blood meal, the bug might remain alive for a year or more, but could not grow or moult. He suggested that the temporary failure of the brain, or another endocrine organ, to secrete a growth-promoting hormone might be the cause of arrested growth in diapausing insects. Although diapause always involves cessation of growth or development, such a unitary theory covering all instances, as suggested by Wigglesworth and propounded by Hinton (1953), seems unlikely. The work of Williams (1947a) and others showed that the neurosecretory cells of the brain, and the thoracic glands, were concerned in the pupal diapause of the cecropia silkworm, Hyalophora (=Platysamia) cecropia. On the other hand, Fukuda (1951, 1952, 1953a,b,c) and Hasegawa (1951) showed that the egg diapause of the silkworm, Bombyx, was controlled by the maternal brain and the neurosecretory cells of the suboesophageal ganglion. The secretion of the latter induced the egg to develop as the diapause-type. Reproductive arrests, characteristic of adult diapause, seem to be controlled by the corpora allata, which, in turn, may be under the control of the brain (Scharrer, 1957).

The brain has been implicated in some way with the initiation, maintenance, or termination of diapause in most insects investigated. In insects with a facultative diapause, temperature and length of or change in the photoperiod are the most common environmental factors that trigger the arrest (Lees, 1952). The evidence suggests that the insect is influenced directly by the cycle of illumination. Tanaka (1950, cited in Lees, 1952) showed that the eyes are not the receptors of the photoperiodic stimulus that triggers diapause in Antheraea pernyi. Complete darkness prevents diapause and short day length promotes it in this insect. However, all larvae in which the lateral ocelli were covered with enamel during the larval period or cauterized in the fourth instar formed diapause-pupae in response to a short day length. It seems likely that the light penetrates the integument and affects the neurosecretory cells of the pars intercerebralis itself (Lees, 1952). In the cecropia silkworm, which has a temperature-influenced arrest, van der Kloot (1955) reported that the loss of endocrine activity in the brain shortly before the pupal moult was paralleled by the disappearance of spontaneous electrical activity and cholinesterase from the brain. Williams (1956) showed that the return of secretory ability to the brain was promoted by low temperatures, and van der Kloot (1955, 1960) suggested that the low

temperatures promoted the accumulation of a cholinergic substance in the brain which triggered the synthesis of cholinesterase. The return of secretory activity to the brain just prior to adult development was paralleled by a return of cholinesterase and electrical activity to the brain. Van der Kloot (1955) suggested that the neurones of the entire brain were inactivated during diapause to ensure the inactivation of the neurosecretory cells.

It has been known for many years that colour change in Crustacea is under hormonal control (Carlisle and Knowles, 1959). A colour change that appears to be under hormonal control can be seen in some insects too. Dupont-Raabe (1951, et. seq.) and L'Helias (1955) showed that colour change in Carausius (= Dixippus) was under the control of the brain and the corpora paracardiaca. Dupont-Raabe (1954a,b, 1956c) demonstrated that the chromatophorotrophic principle was also present, though in lesser concentration, in the suboesophageal ganglion, the ganglia of the ventral chain, and the corpus allatum. Brown and Meglitsch (1940) reported that extracts of insect corpora paracardiaca caused a strong contraction of the red chromatophores in the crustacean Cambarus immunis.

Knowledge of the distribution and cytology of the neurosecretory cells has advanced tremendously in recent years. In 1935 Weyer noticed neurosecretory cells

in the pars intercerebralis of the brain and in other ganglia of the honey bee. Hanström (1938) described similar cells in the pars intercerebralis of Rhodnius. These medial cells are located on the dorsal surface of each hemisphere of the protocerebrum, closely adjacent to the midline. The axons of the medial cells run ventrally and cross over into the opposite hemispheres. They exit from the back of the brain as the nervi corporis paracardiaci I (nervi corporis cardiaci I, nervi corpus cardiaci I) and innervate the corpora paracardiaca. In some insects, this nerve continues through the corpus paracardiacum, into the corpus allatum, e.g., in Bombyx (Arvy, Bounhiol, and Gabe, 1953; Bounhiol, Gabe, and Arvy, 1954). In some groups the corpus paracardiacum is joined to the brain by another nerve, the nervus corporis paracardiaco II. The axons forming this nerve do not cross over, but run to the corpus paracardiacum on the same side (Cazal, 1948; Hanström, 1949). The cell bodies of these axons vary in location in different insects. In some Diptera and Hymenoptera they lie in the pars intercerebralis (Brandenburg, 1956a; Cazal, 1948; M. Thomsen, 1954). In Lepidoptera (Williams, 1947b, 1948), Dermaptera (Lhoste, 1953), Hemiptera (Nayar, 1955, 1956a), Drosophila funebris and D. hydei (Köpf, 1957a), Homoptera, Ephemeroptera, and Brachycera (Cazal, 1948) this lateral group lies outside the pars intercerebralis

between the corpora pedunculata and the optic lobes. Dupont-Raabe (1954a,b, 1956a,b, 1957) found one or two neurosecretory cells in the tritocerebrum of Carausius (= Dixippus). She reported that the axons of these cells run to the corpora paracardiaca via a third pair of nervi corporis paracardiaci (Dupont-Raabe, 1956a).

The secretory granules within the neurosecretory cells give them a unique bluish appearance. This was seen in the axons of these cells in Neuroptera (Arvy, 1956), Odonata, Ephemeroptera, and Tenebrio molitor (Arvy and Gabe, 1952, 1953a,b,c), Leucophaea (Scharrer, 1952b), Calliphora (E. Thomsen, 1954a,b), other Diptera and Hymenoptera (M. Thomsen, 1951, 1954), and Lepidoptera (Arvy, Bounhiol, and Gabe, 1953). Scharrer (1951) noted that this material accumulated in the corpus paracardiacum in Leucophaea. She suggested that this material was stored in the corpus paracardiacum near the lumen of the aorta, and postulated that it was released from here into the blood stream. She also suggested that the corpus paracardiacum served primarily as a storage organ for the neurosecretory substance from the brain. When she cut the nerve to the corpus paracardiacum in Leucophaea, the neurosecretory material from the brain piled up in the nerve proximal to the cut, and the corpus paracardiacum was soon depleted of the stainable material (Scharrer, 1952b). This evidence does not mean, however, that the

corpus paracardiacum functions solely as a storage organ; it may have a secretory function as well, and/or it may modify the cerebral neurosecretory material in some way before releasing it into the vascular system (Wigglesworth, 1954b).

Stages in the secretory cycle of the neurosecretory cells, corresponding to definite stages in postembryonic development, have been reported in some insects. Rehm (1951) described cycles of activity in these cells, which are followed by activity in the thoracic glands, in Ephestia, and Gabe (1954a) stated that each larval stage in holometabolous insects is paralleled by a cycle of secretion in the pars intercerebralis.

Neurosecretory cells have been found in other ganglia of the insectan nervous system, but these accounts are rare in comparison with the many descriptions of these cells in the brain. They have been found in the ventral ganglia of several Lepidoptera (Day, 1940), in Ephemeroptera, Odonata, and Tenebrio (Arvy and Gabe, 1952, 1953a, c), Bombyx (Bounhiol, Arvy, and Gabe, 1953), Blaberus craniifer (Geldiay, 1959), Leucophaea (Scharrer, 1955b), Iphita (Nayar, 1953), and Lucilia caesar (Fraser, 1959). Wigglesworth (1956) mentions that he found them in the thoracic and abdominal ganglia of Rhodnius. Kobayashi (1957, cited in van der Kloot, 1960) reported that there were many more neurosecretory cells in the

suboesophageal, thoracic, and abdominal ganglia of Bombyx than there were in the brain.

Neurosecretion has also been reported in other invertebrate groups; the X-organ - sinus gland system of the Crustacea, especially the Decapoda, has been thoroughly studied (Carlisle and Knowles, 1959). Neurosecretory cells have been reported in Nematoda (Gersch and Scheffel, 1958); Nephtys spp. (Clark, 1955a,b, 1959), Nereis irrorata (Defretin, 1952), and Harmothoe spp. (Korn, 1958) of the polychaete Annelida; Lumbricidae (Brandenburg, 1956b; Hubl, 1953, 1956; Michon and Alaphilippe, 1959; Schmid, 1947) of the oligochaete Annelida; Theromyzon rude (Hagadorn, 1958) of the Hirudinea; and in the Onychophora (Gabe, 1954c), Chilopoda (Gabe, 1952, 1956; Palm, 1956), Araneida (Gabe, 1955; Kühne, 1957, 1959; Legendre, 1953, 1958), Phalangida (Gabe, 1954b), Pycnogonida (Sanchez, 1954), and Mollusca (Gabe, 1949, 1951, 1953b; Sanchez and Pavans de Ceccatty, 1957; Scharrer, 1935). Neurones showing secretory activity have also been reported in all orders of the Vertebrata. In fact, the intercerebralis - paracardiacum - allatum system of insects shows striking morphological similarity to the hypothalamo - hypophyseal system of the vertebrates (Hanström, 1941, 1949, 1953; Scharrer and Scharrer, 1944; Wigglesworth, 1934, 1954a), and to the X-organ - sinus gland system of the Crustacea (Hanström, 1949, 1953;

Carlisle and Knowles, 1959).

From all these studies the fact emerges that cells that combine the structural characteristics of nervous elements with those of gland cells are a common occurrence in invertebrates and vertebrates. They are found in such well known nuclei of the central nervous system as, for example, the pars intercerebralis of the insectan brain, the nucleus preopticus and its homologues of the fishes and amphibians, and the nuclei supraopticus and paraventricularis of the reptiles and mammals. The significance of the occurrence of nerve cells that possess the structural characteristics of gland cells and produce hormones obviously lies in their ability to play the dual role of nerve cells and gland cells, thus providing a mechanism by which these two integrating systems are linked together.

LIFE HISTORY

Hughes (1943) has discussed the history and distribution of A. lineolatus, a European species, in North America since it was first collected on this continent, in Nova Scotia, in 1917. The species was first recorded in Minnesota in 1933, and apparently became an important pest of alfalfa in that state before 1939. It was observed by Mickel "in abundance on alfalfa" in southern Manitoba in 1941 (Hughes, 1943). This insect, which overwinters in the egg-stage, is bivoltine in northern Minnesota and, presumably, southern Manitoba.

The species was not collected in Saskatchewan until 1947, when very occasional specimens were found in one or two alfalfa fields in the Hudson Bay district. Economic infestations did not develop until 1952 when up to three bugs per sweep were found in several fields, but only in the immediate Hudson Bay district. Since 1952, however, it has spread across almost the entire northern agricultural area of Saskatchewan, reaching as far west as Glaslyn, about 70 miles east of the Saskatchewan-Alberta border, by 1958[4].

In the northern agricultural areas of Saskatchewan, hatching from the overwintered eggs in the stems of alfalfa begins in late May or early June, and the nymphal

4. Unpublished field notes, Forage Crop Insects Project, Canada Agriculture Research Station, Saskatoon, Sask.

period lasts until mid-July. There are five nymphal instars. The first two nymphal periods last four days each and the last three five days each, under normal field conditions. Adults begin to lay eggs in early August, and the eggs remain unhatched in the stubble and debris throughout the fall and winter. Thus, it seems that the species has undergone some physiological adaptation to a univoltine life history, in order to survive the short summer season of this area.

MATERIALS AND METHODS

The insects used in this study were collected in the field, in the Nipawin district of Saskatchewan, as first and second instar nymphs in early June. All succeeding stages were reared from this population in the laboratory, under controlled conditions: temperature at 68° ± 2°F.; humidity at 80% ± 5% R.H.; and photoperiod at 16 hours light : 8 hours dark. The insects were kept in plastic boxes, 7 in. X 4 in. X 5 in., fitted with plastic screen tops, and were maintained on fresh alfalfa cuttings and lettuce leaves. Mortality, which is normally about 95% in laboratory reared mirids, was kept down to approximately 65% by changing the food every second day. Freshly moulted nymphs were placed in new cages, so that their ages could be reasonably estimated.

Gross morphology of the central nervous system and associated structures, was determined by dissection of adults, mainly, and fourth and fifth instar nymphs. Methods of dissection were as described for Liocoris unctuosus (Ewen, 1960).

For histological studies, nymphs of each instar were killed and fixed at the following times: just after moulting, approximately in the middle of the intermoult period, and about one day prior to the next moult. In addition, several fourth and fifth instar nymphs were

killed and fixed on each day of the intermoult period. Adults were killed and fixed just after metamorphosis, and every second day thereafter for the next 14-day period. Some adult females were killed and fixed just after oviposition. The insects were killed and fixed in Masson's modification of Bouin's fluid, which uses trichloroacetic acid instead of acetic acid (Foot, 1933), or in Helly's fluid; the last two or three segments of the abdomen were cut away to allow the fixing solution to penetrate easily. The Masson-Bouin fluid gave the best fixation; the use of 0.5 to 1.0 per cent of trichloroacetic acid, instead of acetic acid, reduces the cytolysis often seen in material fixed in other Bouin's fluids that contain five per cent or even two per cent acetic acid. Tissues were fixed 16 to 18 hours and subsequent dehydration followed the usually suggested procedures for these fixatives. The tissues were cleared in benzene, to reduce hardening as much as possible. Infiltration was carried out in three paraffin baths, each of one hour duration, in a vacuum oven at 63°C.; the tissues were then oriented and embedded in 60-63°C. m.p. paraffin. Some insects were double-embedded in celloidin-paraffin, following the method of Péterfi (1921).

Transverse and frontal serial sections were cut at 4 to 7 μ without difficulty, though it has been

stated repeatedly in the literature that it is very difficult, if not impossible, to cut such thin sections of insects embedded in paraffin because of the brittle cuticle. Care had to be taken, however, to ensure that the microtome knife was sharp and free from "nicks". Hand sharpening, with well graded aluminum oxide abrasive in suspension and a lapping technique (Bell, 1958), produced a knife with a smooth facet, no rounding off at the edge, and an edge that was free from "nicks" and gaps and was quite straight. By cutting the sections with a slow, smooth stroke, tearing of the cuticle at the upper edge of the section was avoided. No additional treatment of the tissues or of the paraffin block, such as selective softening agents or special pre-embedding procedures, was found necessary or helpful.

The stain most commonly used in this study was a new aldehyde fuchsin technique (Ewen, 1961); the chrome - alum - haematoxylin and phloxin method of Gomori (1941), which is especially useful for distinguishing the thoracic glands, was also used extensively. Neither of these two methods are histochemically specific for neurosecretion (M.L. Cameron, personal communication), but both demonstrate very clearly the distribution of neurosecretory colloid. Although the new technique (Ewen, 1961) is in press, it has not yet

appeared, and therefore the main points are repeated here. The method incorporates some of Cameron and Steele's (1959) modifications and still uses Halmi's (1952) counterstain, but differs from these and other methods in several important points of procedure.

Preparation of the stain. The aldehyde fuchsin is prepared according to Gabe's (1953c) recipe which greatly improves the poor keeping quality of the stain as originally described by Gomori (1950). The yield of dry dye is about one gram. Make up a stock solution of 0.75 grams of the dry dye in 100 ml. of 70% ethanol. This solution will keep for one year without any apparent change in staining characteristics. The staining solution found best for the insect material used is:

stock solution	25 ml.
70% ethanol	75 ml.
glacial acetic acid	1 ml.

The method is as follows:

1. Remove paraffin and hydrate sections in the usual way.
2. Oxidize, one minute, in acid permanganate (Gomori, 1941).
3. Rinse in distilled water.
4. Decolorize in 2.5% sodium bisulphite.
5. Pass through rinses of distilled water, 30%, and 70% ethanol, to aldehyde fuchsin. Stain 2 - 10 minutes.

6. Wash in 95% ethanol.

7. Differentiate, 10-30 seconds, in acid - alcohol:

Absolute ethanol	100 ml.
concentrated HCl	0.5 ml.

8. Pass through rinses of 70% and 30% ethanol, and distilled water.

9. Immerse, 10 minutes, in phosphotungstic - phosphomolybdic acid:

phosphotungstic acid	4.0 grams
phosphomolybdic acid	1.0 grams
distilled water	100 ml.

10. Rinse in distilled water.

11. Counterstain one hour (Halmi, 1952):

distilled water	100 ml.
light green SF yellowish	0.4 grams
orange G	1.0 grams
chromotrope 2R	0.5 grams
glacial acetic acid	1.0 ml.

The solution keeps indefinitely.

12. Rinse in 0.2% acetic acid in 95% ethanol.

13. Dehydrate rapidly through absolute ethanol; clear in xylol; mount in Canada balsam.

Results: cytoplasm, light green; nuclei, orange; neurosecretion, dark purple; neuropile mass, light green; corpus paracardiacum, green, with the secretion collected there a deep purple; corpus allatum, pale green.

Although prior oxidation with acid permanganate permits considerable reduction in staining time with

aldehyde fuchsin, the sections often take up a faint lilac background stain after this treatment. This is especially noticeable if oxidation is prolonged for more than one minute. However, the background colour can be suppressed by passing the slides quickly through acid - alcohol (step 7), and this treatment in no way affects those structures having an affinity for aldehyde fuchsin.

As Cameron and Steele (1959) emphasize, slides must be washed in alcohol both before and after staining with the aldehyde fuchsin. Otherwise the aldehyde fuchsin may precipitate in regions where selective staining does not occur. The addition of 1.0 ml. of glacial acetic acid to the aldehyde fuchsin, as originally suggested by Gabe (1953c), seems to improve its selectivity, especially in older batches of stain.

It is important that the sections be stained as soon as possible after they are cut. Three- or four-day old sections do not take up the aldehyde fuchsin nearly as well; possibly an auto-oxidation in the air prevents the necessary strong oxidation of older sections in the acid permanganate.

While this method is more time consuming than either Gabe's (1953c) or Cameron and Steele's (1959) procedures, it stains neurosecretory products more distinctly and has the advantage of differentiating other tissues in the sections so that the same slides may be

used for microanatomical studies.

Drawings were made on squared paper, using an ocular grid, previously calibrated with a stage micrometer, in the microscope.

RESULTS

GROSS MORPHOLOGY

As previously mentioned (Ewen, 1960), little information on the nervous system of the Miridae has appeared. The descriptions presented here are of the anatomy and relationships of the glands, ganglia, and main nerve trunks; tracing of the finer nerve branches was not attempted. Terminology employed for the nervous system is based on Snodgrass (1935), and that for the retrocerebral complex is after Cazal (1948).

Imms (1957) reports that the nervous system in Hemiptera is concentrated, with the thoracic and abdominal ganglia to a large extent fused. In A. lineolatus the meso- and meta-thoracic ganglia are fused with the abdominal ganglia into a common centre; the prothoracic and suboesophageal ganglia remain separate and distinct (Figs. 1, 3). The present study revealed little difference in gross morphology between this species and L. unctuosus (Ewen, 1960), as might be expected in two closely related species.

The brain in insects is primarily a connecting centre between the sensory organs of the head and the neurones of the cephalic, thoracic, and abdominal motor centres. The major part of the brain consists of a

neuropile tissue mass which is made up mainly of the proximal ends of nerve tracts, and nerve roots from such structures as the compound eyes, ocelli, sensory parts of the antennae, and the oral cavity. The main function of the brain is to regulate those activities that are not controlled by local centres such as the suboesophageal ganglion and the ganglia of the ventral chain, although the latter are often indirectly controlled by the brain. The motor centres of the brain are in the deuto- and trito-cerebral lobes, with motor connexions with the musculature of the antennae and labrum, and, if any, muscles innervated from the sympathetic system.

PROTOCEREBRUM. The protocerebrum is the largest and most dorsal part of the neuropile mass. It is made up of the lateral protocerebral lobes, the median pars intercerebralis, and the axonal and glomerular masses of the dorsal corpora pedunculata or "mushroom bodies", medio-dorsal pons cerebralis, corpus centrale, and latero-ventral corpora ventralia.

The optic centres are ganglia situated in the optic lobes and are so closely connected with the proto-cerebral lobes that they may be regarded as part of the protocerebrum, although in origin they are distinct. In A. lineolatus the optic lobes are only slightly narrowed at their bases and, therefore, the nervus opticus is very short and stout. There are no nervi ocellari (Fig. 1).

The nervi corporis paracardiaci interna and externa arise from the posterior margin of the protocerebrum, near the median line, and extend to the corpora paracardiaca. The two nerves of each side unite near their points of emergence from the brain, so that they appear as a single nerve throughout almost their entire length; they are distinctly free from the aortic wall (Fig. 2).

DEUTOCEREBRUM. The chief functions of the deutocerebrum are sensory innervation of the antennae and motor supply to the antennal musculature. The two lateral lobes of the deutocerebrum are connected by internal commissure tracts. In A. lineolatus, the nervus antennalis is a long, narrow stalk, arising anteriorly and extending forward and laterad to the antennae, supplying, on the way, small motor branches to the intracephalic antennal muscles (Fig. 1).

TRITOCEREBRUM. A very delicate, external nerve tract, the tritocerebral commissure, connects the two tritocerebral lobes. This very small nerve passes from the ventral margin of the tritocerebrum of one side, ventrally around the pharynx, to the tritocerebral lobe of the other side, and is closely associated with the circumoesophageal connectives.

The nervus labrofrontalis arises more anteriorly from the tritocerebrum and passes dorso-medially to the

latero-dorsal surface of the pharynx where it divides into two branches. The frontal ganglion connective passes anteriorly and medially to the small frontal ganglion, which lies beneath the brain on the dorsal surface of the pharynx. The second branch of the labro-frontal nerve, the labral nerve, extends anteriorly to the labrum, supplying the labral muscles and the area of the clypeus and lower frons (Fig. 1).

The ventral, or circumoesophageal, connectives join the tritocerebrum and the suboesophageal ganglion. The tritocerebral commissure, mentioned above, arises along with the circumoesophageal connectives.

SUBOESOPHAGEAL GANGLION. The suboesophageal ganglion is a dorso-ventrally flattened, somewhat triangular-shaped body, lying in the cervical region. It innervates the mandibular, maxillary, and labial segments, the salivary glandular system, some of the cervical musculature, and the thoracic glands. It also sends a branch to the nervi corporis paracardiaci. As in L. unctuosus (Ewen, 1960), a hypopharyngeal nerve was not seen (Fig. 1).

The nervus mandibularis, nervus maxillaris, and nervus labialis arise on the ventro-lateral sector of the ganglion. The mandibular and maxillary nerves innervate their respective mouth parts with sensory and motor neurones, and the labial nerve extends forward,

where it sends a sensory branch to the labium and a motor branch to the labial muscles. The "nerf laterale", described by Cazal (1948) as branching from the paracardiacal nerve, is quite distinct in A. lineolatus, though it was not seen in L. unctuosus (Ewen, 1960). It is a very delicate nerve, usually branching from the mandibular nerve, although it sometimes has a separate origin from the suboesophageal ganglion, and runs dorsally to enter the nervi corporis paracardiaci just posterior to the union of the nervi corporis paracardiaci interna and externa (Figs. 1, 2).

Two pairs of cephalic nerves, which innervate the majority of the post-cephalic and cephalo-thoracic muscles, and a pair of salivary duct nerves, arise from the postero-ventral margin of the suboesophageal ganglion.

SYMPATHETIC SYSTEM OF THE HEAD. The cephalic sympathetic system is made up of the frontal ganglion and its connectives, the nervus recurrens, and the ganglion hypocerebralis.

The frontal ganglion, joined by the frontal connectives to the tritocerebrum, is a small, slightly bilobed ganglion situated on the dorsal surface of the pharynx. It may give rise to two or more small nerves that innervate the local area of the pharynx. The frontal ganglion is connected with the hypocerebral ganglion via the thin, unpaired recurrent nerve which

extends along the dorsal wall of the pharynx, between it and the aorta. The recurrent nerve enters the hypocerebral ganglion a short distance behind the brain. A single, median oesophageal nerve continues posteriorly from the hypocerebral ganglion (Figs. 2, 4).

RETROCEREBRAL COMPLEX. (Figs. 2, 6). The retrocerebral endocrine system in A. lineolatus includes the paired corpora paracardiaca and a single corpus allatum, lying in the cervical region posterior to the protocerebrum. The paracardiaca are lateral, subaortic bodies, similar in structure to those of L. unctuosus (Ewen, 1960). There is no distinct commissure between the paracardiaca and the hypocerebral ganglion, and apparently the latter is enclosed within the posteromedian margins of the paracardiaca. The nervi corporis paracardiaci and the "nerf laterale" have already been discussed in the descriptions of the protocerebrum and suboesophageal ganglion respectively.

The corpus allatum lies directly behind and is closely applied to the corpora paracardiaca. Allatal nerves are not evident. The corpus allatum is distinctly bilobed, with the left lobe slightly larger than that on the right side, unlike in L. unctuosus (Ewen, 1960) where the corpus allatum is almost globular.

PROTHORACIC GANGLION. The prothoracic ganglion and its nerve branches are quite similar to those of

L. unctuosus. The ganglion itself lies between the front legs. There are two main nerve branches from the ganglion. The anterior nerve innervates muscles in the cervical region and around the bases of the wings; the posterior nerve innervates the muscles of the front legs. These two nerves always have separate origins from the ganglion (Fig. 1).

THORACO - ABDOMINAL GANGLIONIC CENTRE. The remaining thoracic and abdominal ganglia are fused into a common centre lying in the posterior half of the meso-thorax. The nerve branchings in the thorax, which are similar to those in L. unctuosus, are shown in Fig. 3. Innervation of the male reproductive organs is also quite similar to that in L. unctuosus.

In the adult female, there are two pairs of small nerves that innervate the body wall musculature of the anterior abdominal segments, and one large median nerve, from the posterior margin of the thoraco - abdominal ganglion. The median nerve supplies further small branches to the body wall of succeeding segments and to the gut, and terminates in four large branches. The lateral of these terminal nerves on each side innervate the ovaries, calyx, and lateral oviducts, and may send a small branch to the suspensory ligament. The two median terminal nerves innervate the spermathecal gland, seminal depository, common oviduct and genital chamber, with large branches

to the musculature, in the posterior abdomen, that controls the ovipositor (Fig. 3).

The major ganglia are easily recognizable in all stages. Although no measurements were made, it was obvious that there were no major changes in the sizes of these ganglia relative to each other or to the whole insect, throughout the postembryonic period. The ganglionic centres occupy approximately the same positions in all stages, and they seem to be of approximately the same relative sizes.

Likewise, the major axonal and glomerular masses of the protocerebrum and deutocerebrum change little from first instar to adult. The pons cerebralis (Fig. 5, Pncr), the most dorsal of these centres, lies in the posterior part of the pars intercerebralis. It is somewhat dish-shaped, with the concavity downwards. The corpora pedunculata (Fig. 5, Cpd), or "mushroom bodies", are the largest and most obvious of the association centres in the brain. They are located in the dorsal part of the brain, between the pars intercerebralis and the protocerebral lobes. The stalks of the "mushroom bodies" extend inward to the central part of the neuropile, and almost meet below the pons cerebralis and the corpus centrale. The corpus centrale (Fig. 5, Cc) lies slightly anterior to the pons and immediately beneath it. The small corpora ventralia (Fig. 5, Cv) lie in the ventral

part of the protocerebrum, above and slightly medial to the antennal centres of the deutocerebrum. They are connected with each other by a distinct commissure which passes beneath the stalks of the "mushroom bodies". The deutocerebral antennal centres (Fig. 5, AntC) are also connected with each other, via the deutocerebral commissure, which passes beneath the commissure of the corpora ventralia, in the ventral part of the brain.

All parts of the insectan brain are intimately interconnected by many fibre tracts. Only four of these, however, traverse the brain between similar centres, and can thus accurately be called commissures according to Snodgrass' (1935) definition. These four commissures, the first between the ventral bodies, the second between the antennal centres, the third uniting the optic lobes, and the fourth, the tritocerebral commissure, can be seen in all instars of A. lineolatus.

The retrocerebral endocrine glands exhibit considerable variation over the postembryonic period. Again, although no measurements were made, it was obvious that the corpora paracardiaca and, especially, the corpora allata of the first and second instars were considerably smaller, relative to the size of the brain, than they were in any succeeding stage. As will be discussed later, however, the histology of these organs

is quite similar in all life stages. The significance of the relatively small size of the retrocerebral glands to the control of moulting in the early instars is unknown.

HISTOPHYSIOLOGY

THE NEUROSECRETORY CELLS. The concept of neurosecretion has been developed by Ernst and Berta Scharrer (1945). These authors define neurosecretory cells as "--- nerve cells which resemble gland cells in that they show the cytological features of glandular activity, i.e., they produce and discharge granules and colloidlike material". This concept has been the basis for the description of neurosecretion by other authors and will be followed here.

The morphological relationship between the neuroglandular elements and the central nervous system is shown in Fig. 6. Two types of neurosecretory cells, based on size and on selective stainability with the chrome-alum-haematoxylin and phloxin (hereafter abbreviated CAHP) and aldehyde fuchsin (hereafter abbreviated AF) techniques, were seen (Table 1). The most conspicuous type, the Type A cells (Figs. 7-10), measure about 15 to 25 μ in length, and 9 to 13 μ across the broadest part (Table 1). The Type A cells appear teardrop-shaped in some sections, but most often are

irregular in shape. The nucleus is spherical or oval in section, has little chromatin, and has one large nucleolus. The cytoplasm of these cells stains intensely with the light green of the AF technique and with the phloxin of the CAHP technique, but its stainability greatly declines in the presence of the neurosecretory colloid that eventually fills most of the cytoplasm and gives this cell-type its characteristic tinctorial affinities. This neurosecretory colloid stains intense purple with the AF technique and deep blue or blue-black with the CAHP technique. No vacuoles were seen. Neurosecretory colloid can be traced for some distance along the axons of these cells (Figs. 8, 10).

The second type of neurosecretory cells, the Type B cells, are somewhat smaller than the Type A cells, measuring about 10 to 13 μ by 5 to 11 μ (Table 1). The cells are generally irregular in shape, but may appear spherical in some sections. The nucleus is similar to that of the Type A cells. In the Type B cells, the finely granulated cytoplasm stains blue-green with the AF technique and reddish with the CAHP method (Figs. 7, 8, 9).

Type A cells only occur in the pars intercerebralis medialis of the protocerebrum. The Type B cells, however, are found in the pars intercerebralis medialis and lateralis, the prothoracic ganglion, and the thoraco-abdominal ganglionic

centre (Figs. 11, 12).

The pattern of distribution of the neurosecretory cells is similar in both the first and second instar nymphs. The large Type A cells are located in the posterior half of the pars intercerebralis medialis, on either side of the fissure separating the two protocerebral lobes (Figs. 9, 10). There appear to be about five or six of the Type A cells in each lobe of the pars intercerebralis in these early instars, but their number could not be determined with certainty. The axons of the Type A cells form distinct pathways that can be easily traced through the brain. The axons extend forward for a short distance and then cross over at about the level of the central body, so that the axons from the cells in the left side now traverse the right side of the brain and vice versa, and then proceed posteriorly. From their point of exit at the posterior margin of the brain, they proceed, laterad to the oesophagus, to the anterior tip of the corpora paracardiaca. The material in these cells and in their axons is granular and stains purple with the AF technique (Figs. 9, 10).

The number of Type B cells in the protocerebrum of the first and second instar nymphs was difficult to determine. There appeared to be only one or two of these cells, scattered among the Type A cells, in the pars intercerebralis medialis (Fig. 9). In the pars inter-

cerebralis lateralis, which is separated from the pars intercerebralis medialis by the corpora pedunculata, as many as three Type B cells have been seen in each lobe. It was quite difficult to trace the axons of these cells, especially those in the pars intercerebralis lateralis, in these first two nymphal stages. In a few sections some colloid was seen along their axons, but their subsequent pathways could not be determined. Hanström (1949) mentions that the axons of the pars intercerebralis lateralis cells do not cross over but pass directly to the posterior surface of the brain where they form the nervi corporis paracardiaci externa.

No evidence of neurosecretory cells was seen in either the circumoesophageal connectives or the suboesophageal ganglion. In the prothoracic ganglion and the thoraco - abdominal ganglionic centre occasional cells that had tinctorial characteristics similar to those of the Type B cells were seen, but their occurrence was so variable and unpredictable that I hesitate to call them neurosecretory. This situation in respect to the post-cephalic ganglia in the first and second instar nymphs is in marked contrast to the appearance of neurosecretory cells in these ganglia in later stages, as will be described below.

The number and distribution of neurosecretory cells is quite similar in each of the remaining nymphal

stages, and in the male and female adult. The large Type A cells of the pars intercerebralis medialis are again the most conspicuous cell-type in the brain (Figs. 7, 8). There are usually ten of these Type A cells in each lobe of the protocerebrum, and their axons can easily be traced to the corpora paracardiaca, as described above. Usually two Type B cells are also found in the pars intercerebralis medialis, scattered among the Type A cells (Figs. 7, 8). These Type B cells were more difficult to see in the third instar than they were in the later instars, or in the adults. Granular substance was seen for a short distance along the axons of these B cells in a few sections. These axons start out in the same general direction as those from the Type A cells of this region, but they could not be traced as far as the nervi corporis paracardiaci. In the pars intercerebralis lateralis, three of the Type B cells were seen (Fig. 13). The axons of these lateral cells could be followed for about half the distance between the location of the cells and the posterior border of the brain. These axons proceed downward and backward toward the point where the nervi corporis paracardiaci externa exit from the brain.

No neurosecretory cells were seen in the circumoesophageal connectives or the suboesophageal ganglion in any stage. In the prothoracic ganglion of

the third, fourth, and fifth instars, and the adults, six of the Type B cells were seen (Fig. 11). These cells appeared in two groups, of three cells each, one on either side of the postero-lateral portion of the ganglion, along the ventral margin. The cytoplasm of these cells was more or less filled with granules that could be traced for a short distance along the axons. The final destination of these axons could not be determined, however, as was the case with other Type B cells in the central nervous system. The thoraco-abdominal ganglionic centre of these stages contains ten neurosecretory cells of the B type (Fig. 14). The distribution of these cells is shown in Fig. 12. Two cells, one on each side, are at the medio-dorsal side of the ganglion . The other eight cells occur in four groups of two cells each, with four cells on either side of the ganglion. Four of these cells, two on each side, are found dorsally at the anterior edge of the ganglion; the remaining four cells, two on each side, are at the postero-ventral margin of the ganglion (Fig. 14). The axons of these ten cells do not form any distinct pathways and their destination is unknown.

Quite a few cells in various parts of the brain, in the circumoesophageal connectives, and in the ventral ganglia, stain more or less purplish with aldehyde fuchsin, but I do not regard these as neurosecretory. They can

not be identified with the CAHP technique. They occur singly and do not differ in size or in nuclear size from surrounding cells. They usually stain a rather weak purple with the AF, but a few of them stain distinctly. Some of these cells appear to contain granules, but others do not; in no cell, however, was the granulated cell content seen in the axons of the cells. Similarly, material that stains purple with AF may be seen in the neuropile of all the ganglia. These appear as moniliform fibres with purplish swellings along their length, and are especially evident in the tritocerebrum and thoraco-abdominal ganglionic centre (Fig. 15). Whether these fibres represent the axonal transport of neurosecretory material from known, or unknown, neurosecretory cells, could not be determined.

A secretory cycle, representing stages in the physiological activity of the cell, can be seen in both types of neurosecretory cells, but most clearly in the Type A cells, and in these most clearly in the fifth instar and adult stages of the insect (Fig. 7). With the AF technique, the cycle in the A cells appears as follows: the light green-staining cells are probably in a resting stage. The nucleus of the resting cell measures about 5 μ in diameter. The first indications of secretory activity are the appearance of many small, blue-black granules in the cytoplasm, and a marked increase in the

size of the nucleus to 8 to 9 μ in diameter, when the nucleolus is most prominent and stains a bright orange. At this time the cell has reached its maximum size. As the cycle continues, these blue-black granules appear to coalesce into purplish droplets that eventually fill most of the cytoplasm. These secretory droplets can be seen entering the axon of the cell (Figs. 8, 10). The colloid continues to be expelled from the cell, and after this process is completed the cytoplasm once again stains with the light green and the nuclear diameter approximates that of the resting cell.

In addition to the secretory cycle shown by the individual cells, the neurosecretory cells as groups show high and low periods of activity that can be correlated to the physiological stage of development of the insect. This is especially noticeable in the Type A cells, in all stages of the insect's development. Immediately after moulting in the nymphal insect, the amount of stainable colloid in these cells and in the corpora paracardiaca is greatly reduced. The amount of colloid in the Type A cells begins to increase about one day after the moult and continues at a high level until about one day prior to the next moult (Fig. 9). At the peak of activity, the Type A cells show many AF-positive droplets in their cytoplasm and axons, and the corpora paracardiaca are filled with stainable colloid (Fig. 16).

During the last day preceeding the next moult the cells gradually decline in activity and begin to lose their colloid. The corpora paracardiaca, however, remain filled with AF-positive droplets. Perhaps this shut down in activity of the cerebral Type A cells indicates the passing of the critical period of secretion.

The Type B cells, as groups, do not show this cyclical activity in relation to different stages of development in the nymphal insect nearly as clearly as do the A cells. The Type B cells seem to be always filled with phloxinophilic granules. This is not the case in the adult insect, however, where a cycle of activity does occur in some B cells, but it is not as marked nor as clear-cut as that seen in the Type A cells. One interesting point in connexion with the B cells of the prothoracic ganglion, however, was noted. Although no cyclical activity as such was observed in these cells, it was obvious that they contained much more stainable colloid in their cytoplasm during the nymphal period than they did in the adult stages. The significance of this increased activity in these cells during the nymphal period, however, is not known.

In the newly emerged adult female, the Type A

cells are almost devoid of stainable material. During the pre-oviposition period, which lasts about 14 days, the amount of material in the A cells steadily increases, reaching a peak just prior to oviposition (Fig. 8). At this time the abdomen is greatly enlarged, and the ovaries contain many eggs. After oviposition has started, the amount of stainable material in the cerebral A cells is greatly reduced. No such cycle of activity was observed in these cells in the adult male insect.

One further point of interest in the adult female is an apparent cycle of activity in the Type B cells of the thoraco-abdominal ganglionic centre. These ten cells show an increase in volume and in nuclear size, and an increase in the amount of stainable material in their cytoplasm, during about the last two-thirds of the reproductive cycle (Fig. 14). The period of activity in these cells lasts until oviposition has begun. No such cycle of activity was seen in these cells in the male insect. The significance of this activity is not known, but since the only process of growth-and-differentiation going on at this time is concerned with egg-production, it may be that these cells are involved in some way with this process.

Adams and Sloper (1956) have devised a histochemical test that is intended to demonstrate the presence of protein-bound cystine. They applied this test to the

hypothalamo-hypophyseal system of the dog, the rat, and man, and found that the distribution of a cystine-rich material was the same as of the neurosecretory material shown by the usual staining methods. Sloper (1957) later applied this test to the roach Leucophaea and found that here also a cystine-rich material had the same distribution as the neurosecretory colloid. This test was applied to only a few sections of A. lineolatus, and was only partially successful. The results, however, did indicate that a cystine-rich material, with the same distribution as the AF- and CAHP-positive material, is present in this insect.

THE CORPORA PARACARDIACA. The corpora paracardiaca are histologically similar throughout postembryonic development with the possible exception that there are more secreting cells in the fifth instar and adult stages. The gland can be grossly divided into antero-dorsal and postero-ventral parts, each of which is made up of a central area surrounded by a layer two or three cells deep. These cells are of two types, the one about 6 μ and the other about 10 μ in diameter (Table 1). The membrane surrounding the gland is continuous with that of the corpus allatum, and stains a light blue with the AF method.

In the antero-dorsal region of the gland, both the central area and the cytoplasm of the

cells stain with the light green of the AF technique (Fig. 16). The cells in this portion of the gland are about 6 μ in diameter. The nervi corporis paracardiaci enter this portion of the gland, and the many branchings of this nerve can be traced throughout the gland. Some of the branches of this nerve reach the aortic wall, to which the corpora paracardiaca are intimately connected, while others pass through the gland and into the corpus allatum.

In the postero-ventral part of the corpus paracardiacum the cells surrounding the central area are mostly as above, but some larger cells, about 10 μ in diameter, are also seen (Table 1; Figs. 16, 17). These larger cells, which are secretory, stain intensely with the light green of the AF technique and with the phloxin of the CAHP method, and show an accumulation of granular substance in their cytoplasm (Fig. 17). The cells show a type of cyclical activity in that the size of the nucleus increases and decreases in apparent rhythm, but this cycle of activity does not seem to parallel any stage in the activity of the neurosecretory cells. The granular material of these secreting cells of the corpora paracardiaca seems to be released into the central portion of the gland, where it appears as droplets. This material was seen around the aortic wall in a few sections, but in most cases it was masked by the abundant material

from the pars intercerebralis, that accumulates in the gland.

Droplets from the neurosecretory cells of the pars intercerebralis can be traced all along the axons of the nervi corporis paracardiaci (Fig. 16), into the corpora paracardiaca, and through this gland into the corpus allatum. In the later stages especially, this material was clearly seen in the wall of the aorta (Fig. 18). Indeed, in most cases the whole posterior part of the gland appeared to be loaded with these purple-staining droplets from the pars intercerebralis (Figs. 17, 18).

THE CORPUS ALLATUM. The corpus allatum in A. lineolatus is so closely applied to the back of the corpora paracardiaca in all stages that the allatic nerves are not evident exteriorly (Fig. 2). Nervous connexions do exist, however, via the nervi corporis paracardiaci, branches of which pass through the corpora paracardiaca and into the corpus allatum. Covering the gland is a delicate connective tissue sheath that is continuous with the envelope surrounding the corpora paracardiaca. The organ is richly supplied with tracheae, with numerous tracheoles branching over the surface of the gland and among the cells.

The structure of the corpus allatum (Fig. 19) is generally similar in all postembryonic stages. Besides the connective tissue cells of the outer sheath and the tracheae, two types of cells can be recognized in the corpus allatum. The one, perhaps undifferentiated cells, is by far the most numerous. These are small, about 5 μ in diameter, and usually spherical but occasionally irregular in outline. Each has a relatively small nucleus which has little chromatin, and only a small amount of cytoplasm that stains a dull green with the AF technique. Vacuoles or granular substance were not seen in the cytoplasm of these cells (Fig. 20).

The second type are secretory cells. However, they can be distinguished from the "undifferentiated" cells only during active secretory stages of the corpus allatum. When active, the secreting cells are considerably larger, up to about 12 μ in diameter (Table 1), with a large, chromatin-poor nucleus, and an acidophilic cytoplasm (Fig. 20). Secretory granules appear first around the nucleus of the cell and eventually fill the cytoplasm. No intracellular vacuoles were seen, but at the height of activity many intercellular spaces are evident throughout the body of the gland (Fig. 19). The appearance of these spaces suggests that the intracellular secretory material has passed out of the cells into the intercellular spaces. At this time the nucleus has swollen and the nuclear

membrane is much more difficult to discern, but secretory granules were never found within the nucleus. These secretory cells, which are randomly scattered throughout the body of the gland, appear much less frequently than do the undifferentiated cells (Figs. 19, 20).

Cycles of activity in the corpus allatum were most clearly seen in the third and fourth instars. Immediately following the moult to each of the third and fourth instars, both types of cells may be seen in the gland. During about the second day, the secreting cells and their nuclei begin to enlarge, and the amount of granular substance in their cytoplasm increases markedly, reaching a peak on about the fourth day. Thus the start of the secretory cycle in these cells lags behind the cycle of activity in the cerebral neurosecretory cells and behind the increase in activity noted in the thoracic glands. The cytoplasm of the secreting cells is intensely acidophilic at peak activity, due to the numerous secretory granules. Intercellular spaces are also apparent at this time. After the fourth day, or even somewhat earlier in some specimens, the amount of secretory material and the number of intercellular spaces begins to decrease, and the cell and its nucleus begin to decrease in size.

This cycle of activity is also evident in the

fifth instar, but extends over a much shorter time. It reaches a peak on about the third day, or even earlier, and then recedes rather abruptly. In this instar the amount of granular substance in the cytoplasm of the secreting cells appears to be substantially less than during the third or fourth instars, and relatively few intercellular spaces are seen (Fig. 17).

Cycles of secretory activity in the corpus allatum were not nearly as evident during the first and second instars. Secreting cells were seen in the corpora allata, but much less frequently than in later stages. The amount of acidophilic material in the cytoplasm of the secreting cells was rather constant throughout these first two nymphal periods. Intercellular spaces were not observed in the corpus allatum of either the first or second nymphal stages.

In the newly emerged adult female, the corpus allatum appears similar in structure to that at the beginning of the fifth instar. A secretory phase, however, begins almost immediately and a high level of activity is maintained until just before oviposition begins. A marked increase in size of the corpus allatum can be observed during the pre-oviposition period (Fig. 20). This increase in size follows behind the cycle of activity of the Type A cells of the pars intercerebralis. Along with this increase in size, there is

a marked increase in the amount of stainable colloid in the cytoplasm of the secreting cells during the pre-oviposition period, and also an accumulation of neuro-secretory colloid from the pars intercerebralis which has presumably been transported to the corpus allatum via the nervi corporis paracardiaci and the corpora paracardiaca (Fig. 20). In fact, when the Type A cells of the pars intercerebralis reach their peak of activity, the corpus allatum contains a great deal of the purple-staining neurosecretory colloid, which, incidentally, can be traced along the entire length of the axons of the nervi corporis paracardiaci and into the corpus allatum. After oviposition has begun, the corpus allatum is reduced to a size similar to that at the start of the cycle, and the amount of stainable material both from the secretory cells of the corpus allatum itself and from the pars intercerebralis is much reduced.

The corpus allatum in the male adult does not show a marked increase in size similar to that seen in the gland of the female adult. An increase in the acido-philic material of the secreting cells was seen for a short time after emergence, but this never reached the amounts seen in the female gland. Neurosecretory material from the pars intercerebralis was rarely seen in the corpus allatum of the adult male.

THE THORACIC GLANDS. The thoracic glands of

the Hemiptera Heteroptera have been described in some detail by Wells (1954), who mentions that there is little point in continuing to call these organs "prothoracic glands", as they are almost never confined to the prothorax. The glands in A. lineolatus correspond quite closely with Wells' description of these organs in Dysdercus cingulatus.

The thoracic glands are located mainly in the mesothorax in A. lineolatus, extending backward from the prothoracic spiracle (Fig. 4). In a fresh dissection, the gland appears as a long, tenuous, rope-like structure. The structure of the gland can best be seen after treatment with the CAHP method. The gland appears to be almost filled with large, oval nuclei that stain very heavily (Fig. 21). Each nucleus has a single, prominent nucleolus, and is surrounded by a thin ring of cytoplasm. The glands are ensheathed by a thin membrane and are richly supplied with tracheae. No duct was found. No mitotic figures were seen in any nuclei; this agrees with Wells' (1954) findings on other Hemiptera.

Wells (1954) was unable to find any nerves to the thoracic glands of the Hemiptera he examined. In A. lineolatus nerves were found leading from the suboesophageal ganglion to these organs (Fig. 1). In a fresh dissection, the nerve can be seen quite clearly. It extends laterally from the suboesophageal ganglion, just medial to the origin of the maxillary nerve. The nerve

then proceeds laterad around the ventral tracheae and the salivary glands, thence to the thoracic gland . Branches of this nerve can be traced all along the outside of the thoracic gland (Fig. 4).

The thoracic gland nuclei show a cycle of activity during each nymphal instar. Immediately following each moult, the nuclei are at their smallest and the cytoplasm is scanty and does not stain appreciably with the CAHP technique. During the intermoult period, the gland nuclei swell up to about twice their resting size, and the cytoplasm becomes basophilic, staining intense blue with the CAHP method. The most intense staining is seen at the time when the nuclei are at peak size. This occurs about one day prior to the next moult and probably corresponds to the passing of the critical period of activity. The start of this cycle of activity in each nymphal instar appears to be delayed with respect to the cycle of activity of the neurosecretory cells of the pars intercerebralis, but begins before any increase in activity occurs in the corpus allatum.

In the newly emerged adult, the thoracic gland appears similar to any other post-moult gland, but this appearance soon changes. By about the third day after emergence, almost all the glandular cells have disappeared, and the thoracic gland appears to be made up mainly of connective tissue and tracheae. The first

signs of this breakdown can be seen in the one-day old adult, and from then onwards cellular breakdown becomes very conspicuous.

DISCUSSION

Four secretory organs are known to be involved in some way in growth, moulting, and metamorphosis in insects. The neurosecretory cells of the pars intercerebralis of the brain are probably the regulators of the entire endocrine system. Closely associated with these cells are the corpora paracardiaca and corpus allatum. Both these glands are innervated by the cells of the pars intercerebralis via the nervi corporis paracardiaci. The fourth organ is the thoracic gland, which retains integration with the other glands via a nerve connexion to the central nervous system.

Periodic shedding of the cuticle, which allows the growing insect to increase in size, and the differentiation of the body tissues whether in an immature or imaginal direction, are apparently both controlled by the same hormones, as follows: the cycle of secretion by the hormone-producing organs starts in the neurosecretory cells of the brain. These cells produce and secrete a hormone that triggers the thoracic glands to produce and secrete their hormone, ecdysone. Ecdysone, in turn, acts directly on the tissues of the body, setting up a wave of mitoses in the epidermal cells. Shortly after the release of ecdysone in the immature insect, the corpus allatum releases its "juvenile hormone". In the presence of the juvenile hormone the epidermal cells form "larval"

structures, and any other existing "larval" structures are retained. At metamorphosis, however, the juvenile hormone is either absent or present in too low concentration to affect the action of ecdysone, so that imaginal structures are formed and any existing imaginal structures are amplified. After completion of the imaginal moult the thoracic glands break down, thus ensuring that the adult insect shall not moult again. This pattern of secretory activity is essentially similar in both holometabolous and hemimetabolous insects.

The main morphological features of the neurosecretory system of A. lineolatus (Fig. 6) are similar to those of other insects. The most conspicuous groups of neurosecretory cells are in the pars intercerebralis of the protocerebrum. As in many other insects, these cells in A. lineolatus are arranged in two medial groups, the axons of which form the nervi corporisparacardiaci interna, and two lateral groups. The origins of the nervi corporis paracardiaci externa have not been determined with certainty, but they probably come from the lateral protocerebral cells, as has been reported for other Heteroptera (Cazal, 1948; Pflugfelder, 1936).

The presence of three neurosecretory cells in the pars intercerebralis lateralis of each lobe of the protocerebrum agrees with findings in some other insects. For example, Nayar (1955) found three or four lateral neurosecretory cells on each side of the brain in Iphita.

These lateral cells have not been seen at all in some insects (M. Thomsen, 1954) but in most cases where they have been reported they are rather less conspicuous than the medial group (Cazal, 1948; Lhoste, 1953; E. Thomsen, 1952), as in A. lineolatus. In A. lineolatus, these cells were seen in the brain in all instars and, although they did not show any marked cycle of activity, they were active throughout the postembryonic period. Stainable colloid could be seen in these cells, and for a short distance along their axons, in almost all sections. In some other insects these cells are only active during part of the developmental period (Arvy and Gabe, 1953a; Bounhiol, Arvy, and Gabe, 1953; Bounhiol, Gabe, and Arvy, 1954; Lhoste, 1953).

It is rather remarkable that neurosecretory cells were not seen in the suboesophageal ganglion. This ganglion is known to contain neurosecretory cells in many other insects, for example in Iphita (Nayar, 1953, 1955), and cockroaches (Scharrer, 1941). In fact Scharrer (1941) reports that the suboesophageal ganglion is one of the major centres of neurosecretory activity in some roaches.

The presence of neurosecretory cells in the prothoracic ganglion and in the other ganglia of the ventral chain has been demonstrated in other insects. In A. lineolatus these cells in the prothoracic ganglion are more active and have considerably more of the stainable colloid in their cytoplasm during the nymphal period than in the adult stages. Similar results have been reported for the neurosecretory cells of the suboesophageal ganglion in other insects. In the Ephemeroptera and Odonata, in which the thoracic glands are innervated from the suboesophageal ganglion, Pflugfelder (1952) and Arvy and Gabe (1954) have shown that activity in the neurosecretory cells of this ganglion decreases in the adult, where the thoracic glands have atrophied. Continuous activity in the neurosecretory cells of the suboesophageal ganglion during the immature stages has been reported in Bombyx (Bounhiol, Gabe, and Arvy, 1954). These authors conclude that there is a hormonal relationship between these neurosecretory cells and the thoracic glands via the nerves to the thoracic glands.

It has long been postulated that the brain exerts a thoracotrophic action in insects, via a blood-borne hormone. In this case, a nervous connexion

between the brain and the thoracic glands is not necessary for this trophic action. However, Possompès (1953) demonstrated that a nervous connexion to the central nervous system was necessary to enable the thoracic glands of Calliphora to be activated. His results have not been demonstrated in other insects, but the possibility of a nervous stimulation should not be disregarded entirely. Potter and Loewenstein (1955) and Carlisle (1957) have demonstrated that neurosecretory cells are capable of transmitting nervous impulses. The present data are inadequate to support the hypothesis that the neurosecretory cells of the prothoracic ganglion have any thoracotrophic function in A. lineolatus, but this possibility should not be overlooked in future studies on the functional significance of these cells.

The marked increase in the activity of the neurosecretory cells of the thoraco-abdominal ganglionic centre during the last two-thirds of the pre-oviposition period in the adult female suggests that these cells may be involved in some way with the physiology of reproduction. At the time that these cells begin their activity there are no well-formed eggs in the ovarioles, but egg production has begun. Hence it is not likely that these cells are concerned with the beginnings of egg production or with yolk deposition. The increased activity in these cells at this time may be due to some stimulation received from the gonadotrophic hormone of the corpus allatum, which is hyperactive at this time in the adult female. This explanation is not too attractive, however, as no other neurosecretory cells react in this way at this time. It would be interesting to compare these neurosecretory cells in the Saskatchewan population of A. lineolatus with those of the summer generation of the bivoltine population of Minnesota, to determine if perhaps these cells do play some role in reproductive physiology in the adult female, or if they are involved in some way with diapause, which intervenes during the egg stage in this species.

The marked cycle of activity so clearly seen in the A cells of the pars intercerebralis corroborates observations made by other workers on many other insects. Increased activity in the cells begins just after moulting and metamorphosis, and before activity can be noted in any other of the endocrine organs. It is obvious that the whole cycle of secretion in the endocrine system is triggered by these cerebral neurosecretory cells, but the trigger for these cells is not known. In some insects the stimulus to set the moulting cycle in motion is known to be nervous but in most insects it is not known.

It is very difficult to correlate the types of neurosecretory cells observed in A. lineolatus with those described by most other authors, but they can be compared with the A and B cells described by Nayar (1955) in Iphita, although his descriptions are based only on the CAHP technique. The A and B cells in A. lineolatus show the same tinctorial characteristics with the CAHP method as do the corresponding cells in Iphita. Köpf (1957b), using both aldehyde fuchsin and chrome-alum-haematoxylin and phloxin, describes A and B cells in the brain of Drosophila spp. The Drosophila A cells stain purple and the B cells green with aldehyde fuchsin, as in A. lineolatus. But the main problem in A. lineolatus is whether or not the Type A and B cells are mearly phases in the secretory cycle of a single type of cell. Nayar

(1955) suggests that the red B cells in Iphita are merely A cells that have discharged their stainable colloid. The idea that the phloxinophilic cells were only phases in the secretory cycle of the purple-staining cells was proposed earlier by Mathias Thomsen (1954), who observed both red- and blue-staining material within the same cell. This may be the case in the pars intercerebralis medialis of A. lineolatus, where both types of cells occur and it is not possible to say that the identical cell appears as Type B in all sections. But this idea does not seem to hold for the Type B cells of the pars intercerebralis lateralis and of the other ganglia. In the latter Type B cells, there was never any appearance of a blue-staining material in any section, i.e., no intermediate type of cell was seen, and so a transformation of Type A to Type B, or vice versa, seems unlikely in these locations. Further, there is little doubt that both the Type A and Type B cells of A. lineolatus are neurosecretory, according to the Scharrers' (1945) definition. All cells of both types contain granules that stain characteristically and that can be traced for at least some distance along the axons. These criteria correspond to cell types that have been regarded as neurosecretory in other insects and other animals.

Lack of histochemical specificity, an important disadvantage of both the CAHP and AF techniques, should not be overlooked when describing neurosecretory systems

based on staining reactions with these two techniques. This difficulty has been partially overcome by the development of a histochemical test for protein-bound cystine by Adams and Sloper (1956). These authors note that neurosecretory colloid has a high cystine content, but that it is not known if this cystine-rich material is related to the active substance or to the carrier substance of the neurosecretory material. Sloper (1957) extended the studies, which were originally on vertebrates, to include insects. He found that the cystine-rich material as shown by the performic acid-alcian blue technique had the same distribution in the pars intercerebralis - corpus paracardiacum of Leucophaea as did the substance stained by the conventional methods. These results have been corroborated here and in Tenebrio (M.L. Cameron, personal communication). These results lend further support to the contention that both the Type A and B cells in A. lineolatus are neurosecretory, and they also suggest a certain degree of chemical similarity between the neurosecretory substances of vertebrates and of insects.

On the other hand, the appearance of isolated cells that stain more or less with aldehyde fuchsin in various ganglia of the nervous system poses a problem of a different nature. If these cells are regarded as neurosecretory, then we are forced to conclude that a very considerable number of neurones in the central nervous system

are capable of neurosecretion. The fact, however, that these cells cannot be recognized by the CAHP technique disputes this hypothesis, but even more significant is the fact that granules were never seen entering the axons of any of these cells. Thus I do not regard these as neurosecretory in the classical sense. Results of this nature have been encountered by other authors. Single cells or groups of cells exhibiting the characteristics of neurosecretion have been reported scattered throughout the nervous system of insects (Thomsen, 1951), and Clark (1956) reports that about three-quarters of the brain of Nephtys sp. is made up of neurosecretory cells. Of further interest in this regard is the work of Schmid (1947). He found that the number of nerve cells capable of secretion could be substantially increased by treating earthworms with novocaine. He concludes from these results that probably all of the ganglion cells in the earthworm are capable of secretory activity. And Clark (1956) considers neurosecretory cells as only primitive neurones. It may be, then, that these isolated cells that react weakly with aldehyde fuchsin in A. lineolatus are cells that have almost lost their secretory ability. A similar situation exists regarding the purple-staining material that appears in the form of beadlike-swellings in the neuropile of the ganglia. These moniliform fibres were especially evident in the tritocerebrum and the last ganglion. On the basis of Malhotra's (1956) evidence that Bouin's fluids dissolve out the mitochondria in insect axons, these swellings

obviously are not due to mitochondria. The presence of these structures in the last ganglion might be explained by assuming they represent the axonal transport of neurosecretory material from the Type B cells of this ganglion, as the axons of these cells could not be traced for any appreciable distance. But the fact that the material in the neuropile stains purple with aldehyde fuchsin, while the cells themselves stain light green with AF and red with the CAHP technique, tends to contradict this hypothesis. On the other hand, it has been observed in some crabs that the staining characteristics of neurosecretory colloid change from blue-black to red during its migration along the axons (Carlisle, 1953; Matsumoto, 1956). This has not been observed in any insect to date.

The abundance of purple-staining material in the neuropile of the tritocerebrum is more interesting. A nerve branch from the tritocerebrum to the corpora paracardiaca is being more frequently reported as more insects are investigated. Dupont-Raabe (1956a) observed this tritocerebral branch in Carausius (=Dixippus) and she named it the "nervus corporis cardiaci III". Cazal (1948) reports a nerve between the tritocerebrum and the corpus paracardiacum in the Mecoptera, and Englemann (1957) found it in Leucophaea. Although the stainable material in the tritocerebrum of A. lineolatus could not be traced to any nerve, it is tempting to suggest that it leads to a tritocerebral - corpus paracardiacum nerve. It is likely that

this third branch between the brain and the corpus paracardiacum - allatum, like the nerve to the thoracic glands, exists in all insect species.

The transport of neurosecretory material from the neurosecretory cell bodies along their axons to storage-release organs has long been recognized in both vertebrates and invertebrates. Perhaps the most convincing demonstration of this process in insects was that by Scharrer (1952b) in Leucophaea. She reported that if the nervi corporis paracardiaci were cut, there was an accumulation of the stainable neurosecretory material in the nerve proximal to the cut and in the cerebral neurosecretory cells, and that the corpus paracardiacum was soon depleted of the stainable material. She concluded from these results that the corpus paracardiacum functions chiefly as a storage-release organ for the neurosecretory colloid from the brain in insects. The histological picture in A. lineolatus, as in many other insects, also indicates a transport of material from the pars intercerebralis to the corpora paracardiaca and corpus allatum, via the nervi corporis paracardiaci. The fibres of this nerve were never observed to end in the corpus paracardiacum in A. lineolatus, so whether or not this gland is actually innervated by these fibres is not known. The stainable colloid in the nervi corporis paracardiaci could be seen throughout the posterior part of the gland, however, so probably some of the branches of this nerve do end in the corpus paracardiacum itself. This stainable material

was seen in the wall of the aorta in A. lineolatus; in only a few cases (Cazal, 1948; Nayar, 1956a) has this been reported in other insects. The nervi corporis paracardiaci branch profusely throughout the corpora paracardiaca and some of the fibres reach the aortic wall, although branches of the nerve were not seen actually entering the wall of the aorta. The stainable material in the aortic wall, however, appears similar to the granules in the neurosecretory cells themselves.

It is generally assumed that this material is released from the wall of the aorta into the blood, but the actual presence of this substance in the lumen of the aorta has been observed in only a few cases (Herlant-Meewis and Paquet, 1956). Nayar (1956a) describes the presence of neurosecretory material in the wall of the aorta in Iphita but does not mention it in the lumen. In A. lineolatus neurosecretory material was never seen in the lumen of the aorta, but the morphological evidence that the material is distributed in the wall of the aorta can probably be interpreted to indicate its eventual release into the lumen, as has been done with other insects (Cazal, 1948; Nayar, 1956a; Scharrer, 1952b).

Much less is understood about the place of the corpora paracardiaca than of any other gland in the endocrine control of physiological processes in insects. There are many opinions as to the best explanation of the many histological observations of this organ. De Lerma (1933) was the

first to suggest that these organs contained glandular elements, and Pflugfelder (1937) and Cazal (1948) reported glandular cells in the corpora paracardiaca of some Heteroptera. Nayar (1956b) observed large cells with granular cytoplasm in the corpora paracardiaca of Iphita. Secretory cells were also observed in the corpora paracardiaca of A. lineolatus, distributed around the periphery of the postero-ventral part of the gland. The granular cytoplasm of these cells stains green with the AF method and reddish with the phloxin of the CAHP method, and is probably released into the central portion of the gland and thence to the aorta. In most cases this material from the cells of the corpus paracardiacum itself was masked by the accumulation of neurosecretory material from the pars intercerebralis. Thus the corpus paracardiacum in A. lineolatus seems to have two functions; it produces its own hormone as well as acting as a storage organ for the cerebral neurosecretory material.

Very little is known of the functional significance of the corpora paracardiaca. Extirpation experiments involving this gland invariably interrupt the nervous connexions between the brain and the corpus allatum, and so results of such procedures must be interpreted with caution. Vogt (1946), however, observed a delay in pupation when corpora paracardiaca from adults were implanted into last instar Drosophila spp. larvae, and Bodenstein (1953) showed that the corpora paracardiaca are necessary to maintain the thoracic glands in Periplaneta. Cameron (1953)

reported that an active substance produced by the corpora paracardiaca increased the frequency of movements by the Malpighian tubules and increased the rate of heart beat in Periplaneta. And Brown and Meglitsch (1940), among others, reported that extracts of the corpus paracardiacum are active on the chromatophores of many Crustacea. The results obtained here, of course, add nothing to the very complex and poorly understood problem of the functional significance of the corpus paracardiacum, but they do add to the somewhat scanty reports on the presence of secreting cells in this gland.

Cazal (1948) found a lateral nerve from the suboesophageal ganglion to the nervi corporis paracardiaci in the Cryptocerata, but could not identify any connexions with the suboesophageal ganglion in the Gymnocerata. There is no doubt that the lateral nerve found in A. lineolatus corresponds to Cazal's (1948) "nerf laterale", but the significance of this nervous connexion is unknown. It is important, however, to recognize the presence of these nervous connexions if any attempts are made to determine how the activity of the innervated organs is controlled.

The corpus allatum in A. lineolatus has a similar histological structure to that of other insects investigated. The presence of distinct cell borders agrees with observations by Pflugfelder (1937) and Wigglesworth (1934) on other Heteroptera, but Nayar (1954) reports that the corpus allatum is syncytial in Iphita. The presence of cells showing

cytological evidence of secretory ability, and the presence of neurosecretory material from the pars intercerebralis via the nervi corporis paracardiaci, in the corpus allatum, is not new and merits no further discussion here. Similar observations have been made on many other insects (e.g., Arvy, Bounhiol, and Gabe, 1953; Arvy and Gabe, 1953c; Bounhiol, Arvy, and Gabe, 1953; Brandenburg, 1956a; Nayar, 1954, 1956a).

The functional significance of the corpus allatum is much better understood than is that of the corpus paracardiacum. The corpus allatum is known to be the source of a gonadotrophic hormone in some adult insects, and the source of the juvenile hormone in immature insects. In A. lineolatus a marked cycle of activity in the secreting cells of the corpus allatum, which presumably produce the juvenile hormone, was observed. This cycle lags behind the cycle of activity in the neurosecretory cells of the pars intercerebralis, and also behind the increased activity noted in the thoracic glands. This results agree with observations in many other insects. It seems that the cycle of activity is started in the cerebral neurosecretory cells and that these activate, in turn, the thoracic glands and the corpus allatum. Since the increased activity in the cells of the corpus allatum occurs after the cycle of activity has begun in the thoracic glands, it is likely that the juvenile hormone modifies the action of the thoracic gland hormone in an immature direction.

The corpus allatum is active for a much shorter

period in the fifth instar nymph of A. lineolatus than in the preceeding instars, and apparently stops secreting in this stage. Similar changes in the behavior of the corpus allatum have been observed in other insects (Fukuda, 1944; Pfeiffer, 1939; Pflugfelder, 1937; Rehm, 1951; Scharrer, 1946a; Wigglesworth, 1936). Bodenstein (1953) suggested that instead of the juvenile hormone-producing cells of the corpus allatum ceasing activity in the last stage nymph, they are merely over-ridden by the high concentration of thoracic gland hormone. However, this view has not been supported by experimental results. For example, Fukuda (1944) found that implanting as many as three activated thoracic glands would not overcome the juvenile hormone present in young silkworm larvae.

Holometabolous and hemimetabolous insects seem to differ somewhat in their response to the juvenile hormone. At each moult, the hemimetabolous nymph shows slightly more differentiation toward the adult form, whereas there does not appear to be morphological differentiation toward adult form during the larval life in the holometabolous insect. It may be that when the juvenile hormone is present in excess of a certain titer in the holometabola, larval characters of a constant form are expressed. On the other hand, Wigglesworth (1936) expressed the opinion that the gradual appearance of adult characters in the hemimetabola is due to a gradually decreasing concentration of juvenile hormone. He showed that by implanting

the corpus allatum from a third instar nymph into an allatectomized fourth instar nymph, the level of juvenile hormone in the fourth stage was raised above that normally seen in this stage. This sort of relationship apparently does not occur in the holometabola where the actual amount of juvenile hormone present does not seem to matter as long as a certain titer is maintained.

Our knowledge of the functional significance of the endocrine organs is based to a large extent on a correlation between secretory activity in these structures and other physiological events in the life cycle of the insect. This is perhaps especially true of the corpus allatum and the role it plays in reproductive physiology. We have already noted that there is a marked increase in secretory activity in the cerebral neurosecretory cells during the pre-oviposition period, and in the neurosecretory cells of the last ganglion during the last two-thirds of the pre-oviposition period, in the adult female of A. lineolatus. I have suggested that these cells may play a part in reproductive physiology. The role of the corpus allatum must also be considered in discussing endocrines and reproduction. A marked increase in size of the corpus allatum, beginning shortly after emergence and lasting until just before oviposition was observed in the adult female of A. lineolatus. This increase

in size was accompanied by a marked increase in the amount of stainable colloid in the cytoplasm of the secreting cells of the corpus allatum, and also an increase in the amount of neurosecretory material from the pars intercerebralis accumulating in the corpus allatum. This increased activity of the corpus allatum was delayed in relation to activity in the cerebral neurosecretory cells. Also, the pars intercerebralis cells shut down activity later, at the close of the reproductive cycle, than do those of the corpus allatum. Thus it seems that secretory activity in the corpus allatum is triggered by the neurosecretory cells of the pars intercerebralis, but that some other factor may be involved in shutting down activity in the corpus allatum just before oviposition begins.

The fact that the corpus allatum is hyperactive during the pre-oviposition period indicates that it is involved in some way with reproductive physiology in adult females of A. lineolatus. Yolk-deposition and subsequent ripening of the eggs of insects have been demonstrated to be under the control of the corpus allatum in several species (Bodenstein, 1954). Removal of the corpora allata, resulting in atrophy of the eggs due to failure in yolk-deposition, has been reported in Rhodnius (Wigglesworth, 1936), Melanoplus (Pfeiffer, 1939, 1940), Calliphora (Thomsen, 1940), Drosophila spp. (Vogt, 1943a), and Leucophaea (Scharrer, 1946b). Thomsen (1948, 1952) has shown that the cerebral neurosecretory cells are also

involved. If these cells are removed from very young Calliphora females egg development is prevented; and if they are transplanted from mature females egg development starts again. In Carausius (=Dixippus), however, removal of these medial neurosecretory cells did not prevent development of viable eggs (Dupont-Raabe, 1954b). However, Herlant-Meewis and Paquet (1956) suggested that perhaps there was enough neurosecretory material stored in the corpus paracardiacum in this insect that the removal of the medial cells had no marked effect on egg development.

Nayar (1957, 1958) found that the cerebral neurosecretory cells declined in activity during the latter part of the oviposition period in Iphita, but increased again as oviposition began. He also reports that the corpus allatum stops secreting during oviposition in Iphita. He postulates that the neurosecretory cells of the pars intercerebralis trigger the corpus allatum to produce a gonadotrophic hormone early in the pre-oviposition period, and that at the time the eggs are ready to be laid the ovaries release an ovarian hormone that inhibits the corpus allatum and at the same time promotes further neurosecretory release. This new burst of activity in the neurosecretory cells stimulates oviposition. Unlike in Iphita (Nayar, 1957, 1958), the cerebral neurosecretory cells in A. lineolatus were active throughout the pre-oviposition period. It was noted, however, that the

corpus allatum ceases activity just prior to oviposition, and this agrees with Nayar's findings. Thus his hypothesis can be applied, at least in part, to A. lineolatus. The fact that the corpus allatum decreases in activity before oviposition begins, but at a time when the cerebral cells are still active, indicates the action of some other agent on the corpus allatum. Nayar's (1957, 1958) "ovarian hormone" may be this factor. Presumably it is released by the ovaries at the time that the eggs are ready to be laid. Its function in A. lineolatus is not likely to be involved with stimulating neurosecretion, which is active at this time anyway, as much with inhibiting the secretion by the corpus allatum.

The marked increase in size and secretory activity of the corpus allatum evident in the adult female was not seen to nearly the same degree in the adult male of A. lineolatus. Similar observations have been made with other insects. The testes do not seem to need the corpus allatum hormone to function normally. Attempts to disturb spermatogenesis by extirpation of the corpora allata produced negative results in Rhodnius (Wigglesworth, 1936), Calliphora (Thomsen, 1943), and Leucophaea (Scharrer, 1946b). Also no effect on the corpora allata was observed following castration of males of Lucilia spp. (Day, 1943) or Leucophaea (Scharrer, 1946b), whereas castration usually leads to hypertrophy of the corpora allata in female insects (Day, 1943; Pfeiffer, 1940;

Thomsen, 1940, 1943). Apparently there is no evidence that the corpus allatum is necessary for reproductive activity in the male insect.

The functional inter-relationship, if any, between the corpora paracardiaca and the corpus allatum is not well understood. Joly (1945) showed that the corpora allata atrophied after the removal of the corpora paracardiaca in Dytiscus spp., and Scharrer (1952b) reports hypertrophy in the corpus allatum of Leucophaea after section of the nervi corporis paracardiaci. However, these results may be due only to the interuption of the nervous connexions between the brain and the corpus allatum, which invariably accompanies such surgical procedures, as pointed out by Joly (1945). On the other hand, Bodenstein (1953) has shown that the maintenance of the thoracic glands in the immature insect is dependant on an inter-relationship between the thoracic glands and the corpus paracardiacum - allatum system. Wigglesworth (1954a) reports that the thoracic glands break down only when they experience certain special humoral conditions, which are present just prior to the imaginal moult and in the adult insect. In A. lineolatus we have seen that the amount of juvenile hormone is much reduced, if any is present at all, just before the adult moult, due to a marked decline in the activity of the corpus allatum in the last instar. This observation has also been reported

in other insects. Removal of the corpus allatum at this time has no affect on normal metamorphosis (Bounhiol, 1938; Scharrer, 1946a; Wigglesworth, 1954a). In the adult A. lineolatus, especially the female, the corpus allatum is again active, in fact more active than at any other time, shortly after emergence, and thus there must be a much greater ratio of corpus allatum : corpus paracardiacum hormone than at any other time. This overabundance of the corpus allatum hormone may be the cause of breakdown in the thoracic glands. These latter organs were presumably maintained in the nymphal insect by the higher titer of corpus paracardiacum hormone relative to corpus allatum hormone.

The relationship between the thoracic glands and the neurosecretory system has already been discussed. It should be mentioned again, however, that the start of cyclical activity in the thoracic glands in nymphal A. lineolatus was observed to lag behind the cycle of activity in the neurosecretory cells of the pars intercerebralis. This shows again the importance of the cerebral cells as triggers for the whole endocrine system.

The presence of a nerve supply to the thoracic glands in A. lineolatus is of particular interest. It is probable that nerves to the thoracic glands occur in all insects. Lee (1948) found these nerves in lepidopterous larvae and Scharrer (1948) found them in Leucophaea. The ventral glands of Ephemeroptera and Odonata, which

are presumably homologous with the thoracic glands of other insects, also have a nerve supply (Pflugfelder, 1947). It is possible that Wigglesworth (1952a) and Wells (1954) overlooked these nerves in Hemiptera that they investigated.

SUGGESTIONS FOR FUTURE WORK

Preliminary studies of this nature always uncover more problems than they solve, and this study is no exception. The work points out the need for future studies on endocrinology of this species and of insects generally. I have noted some of these needs in previous sections of the paper; the following problems merit special attention.

The most immediately obvious follow up of this work would, of course, be a histological comparison of the neurosecretory cells of the Saskatchewan population with those of the non-diapause summer generation and the diapause winter generation of the Minnesota population.

Further work is needed on the functional significance of all the groups of neurosecretory cells, especially the Type B cells. Except for the cells in the pars intercerebralis medialis in a few species, there is very little known about the functional sig-

nificance of the different groups of neurosecretory cells in insects.

There is also a need for further work on the corpus allatum and its role in reproduction. Allatectomy, castration, and transplantation might provide further information on the interaction among the corpus allatum, the neurosecretory cells of the pars intercerebralis, and the reproductive organs.

The role played by the corpora paracardiaca in insect endocrinology is still poorly understood. Secretory cells have been reported in this gland in a few species, but the functional significance of these cells is virtually unknown. There is some evidence that the activity of the corpora paracardiaca depends on stimulation by the brain. Further work on the inter-relationship between the corpora paracardiaca and the other endocrine centres is needed. Cauterizing or extirpating the medial or lateral cerebral neurosecretory cells, or both groups together, either unilaterally or bilaterally, and observing effects of such procedures on the corpora paracardiaca, may lead to the solution of some of these problems. The "nerf laterale" should also be investigated in this regard. Nervous connexions such as this must not be overlooked in attempting to determine how the activity of the

innervated organ is controlled.

The relationship between the corpora paracardiaca and the thoracic glands is especially interesting in view of Bodenstein's (1953) contention that the thoracic glands are sustained in the nymphal insect by the corpora paracardiaca. In A. lineolatus the very marked increase in the activity of the corpora allata in the adult stages, especially the female, may be of significance in this regard. Perhaps the very high titer of corpus allatum hormone relative to corpus paracardiacum hormone leads to the breakdown of the thoracic glands in the adult insect. It would be interesting to implant several corpora paracardiaca into newly moulted adults that had been allatectomized, to see if the thoracic glands could be maintained under these conditions.

As far as I know, no one has sectioned, in vivo, the nerve leading to the thoracic glands. Since the activation of this organ, and its relationship with the corpora paracardiaca, are not thoroughly understood, such work is of particular interest. The role of the various groups of neurosecretory cells should also be investigated in this regard. Cauterizing techniques would immobilize these cells without drastically altering the existing nervous pathways, and thus their effects, if any, on the thoracic glands could be observed.

Additional morphological work on other insect species should be undertaken to determine if the nerve to the thoracic gland is, as I suspect, of general occurrence in insects.

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TABLE 1

AND

PLATES

TABLE 1

Characteristics of Secretory Cells of the Central Nervous System and Retrocerebral Glands of *A. lineolatus*[1]

Secretory Cell Type	Staining Reactions CAHP	AF	Cell Diameter
neurosecretory Type A	blue-black	purple	15-25 μ X 9-13 μ mean 21 μ X 10 μ
neurosecretory Type B	reddish	blue-green	10-13 μ X 5-11 μ mean 12 μ X 9 μ
corpus allatum	reddish	blue-green	6-12 μ mean 10 μ
corpora para-cardiaca	reddish	greenish	9-12 μ mean 10 μ

1. Based on examination of 180 specimens, chiefly 4th and 5th instars and ♂ and ♀ adults.

PLATE I.

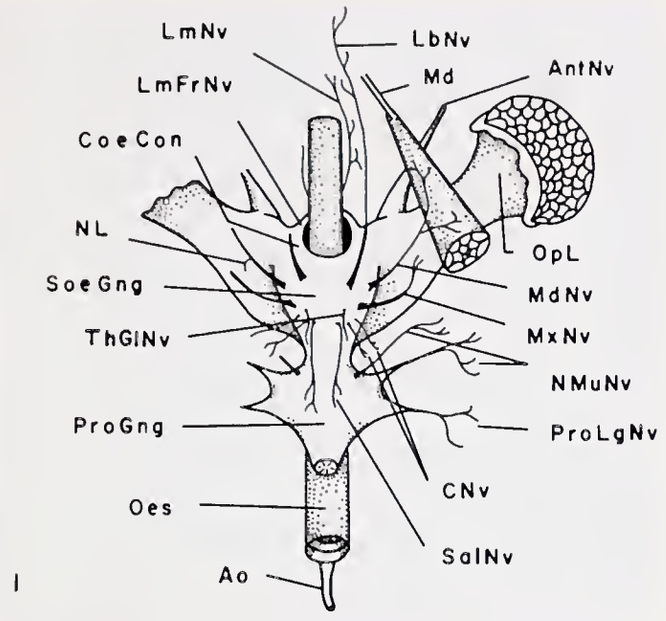
Fig. 1. Diagram of head nervous system of adult A. lineolatus in ventral view. AntNv, antennal nerve; Ao, aorta; CoeCon, circumoesophageal connective; CNv, cervical nerves; LbNv, labial nerve; LmFrNv, labro-frontal connective; LmNv, labral nerve; Md, mandible; MdNv, mandibular nerve; MxNv, maxillary nerve; NL, nerf laterale; NMuNv, neck muscle nerves; Oes, oesophagus; OpL, optic lobe; ProGng, prothoracic ganglion; ProLgNv, prothoracic leg nerves; SalNv, salivary gland nerve; SoeGng, suboesophageal ganglion; ThGlNv, thoracic gland nerve.

Fig. 2. Diagram of retrocerebral complex of adult A. lineolatus, dorsal view. Ao, aorta; CAl, corpus allatum; CPar, corpus paracardiacum; HyGng, hypocerebral ganglion; NL, nerf laterale; NPar, nervus corporis paracardiaco; NParExt, nervus corporis paracardiaco externa; NParInt, nervus corporis paracardiaco interna; Oes, oesophagus; OesNv, oesophageal nerve; RecNv, recurrent nerve; 1 Br, protocerebrum.

Fig. 3. Diagram of abdominal nervous system of adult female A. lineolatus, ventral view. AbNv, abdominal segment nerves; Clx, calyx; HLgNv, metathoracic leg nerve; Lg, suspensory ligament; MedNv, median nerve; MLgNv, mesothoracic leg nerve; Odc, common oviduct; Odl, lateral oviduct; Ov, ovary; Ovl, ovarioles; PoMuNv, posterior abdominal muscles nerve; ThAbGng, thoraco-abdominal ganglionic centre; ThFlNv, thoracic flight muscle nerves; SemDep, seminal depository; SptGl, spermathecal gland; 1 Vl, first valvula; 3 Vl, third valvula; 2 Vlf, second valvifer; VIII St, eighth sternite.

Fig. 4. Diagram showing location of thoracic glands in fifth instar A. lineolatus nymph, dorsal view. FrGng, frontal ganglion; LmNv, labral nerve; Oes, oesophagus; RecNv, recurrent nerve; SalGl, salivary glands; ThGl, thoracic glands; ThGlNv, thoracic gland nerve; Tra, tracheae; 1 Br, protocerebrum.

Plate I.


LmNv
LbNv
LmFrNv
Md
AntNv
CoeCon
NL
OpL
SoeGng
MdNv
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NMuNv
ProGng
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CNv
Oes
SalNv
Ao
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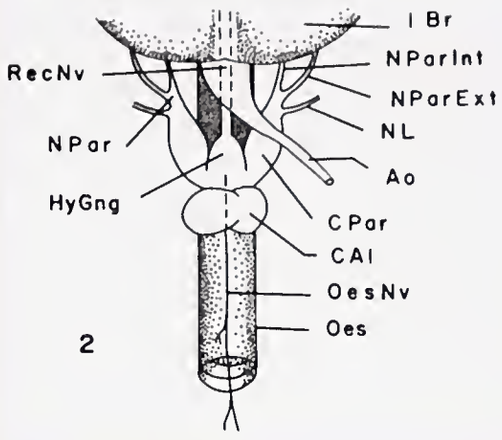
I Br
RecNv
NParInt
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NL
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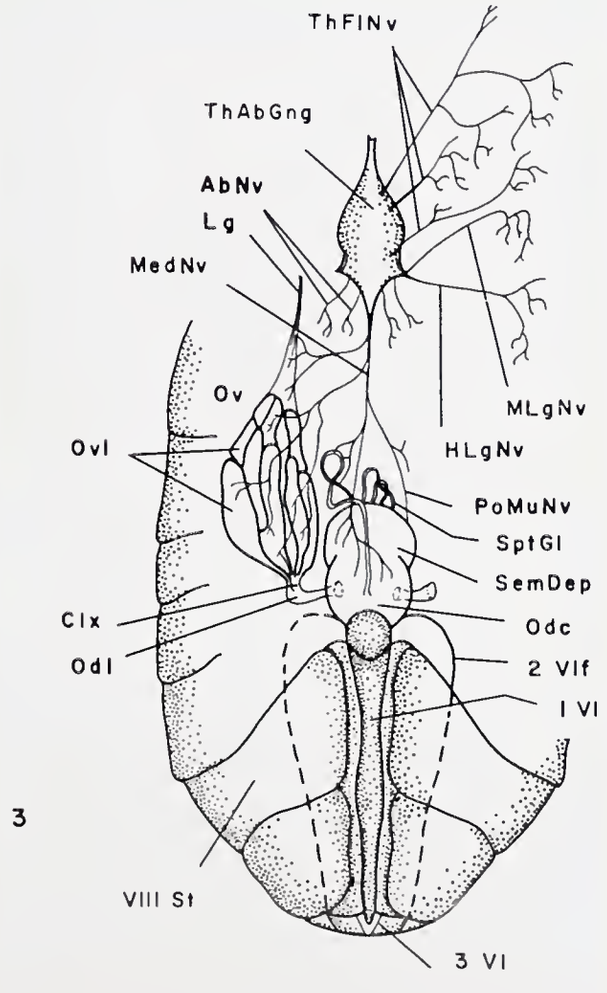
HyGng
CPar
CAl
OesNv
Oes
2


ThFlNv
ThAbGng
AbNv
Lg
MedNv
Ov
MLgNv
Ovl
HLgNv
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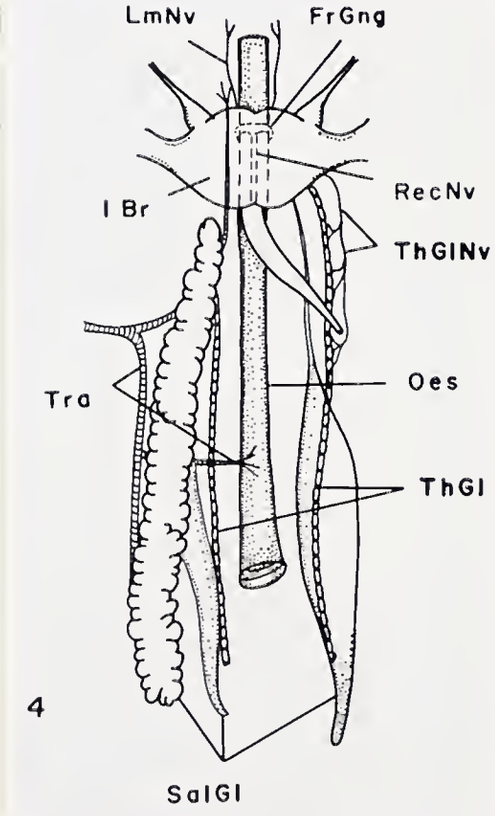
I Vl
3
VIII St
3 Vl


LmNv
FrGng
I Br
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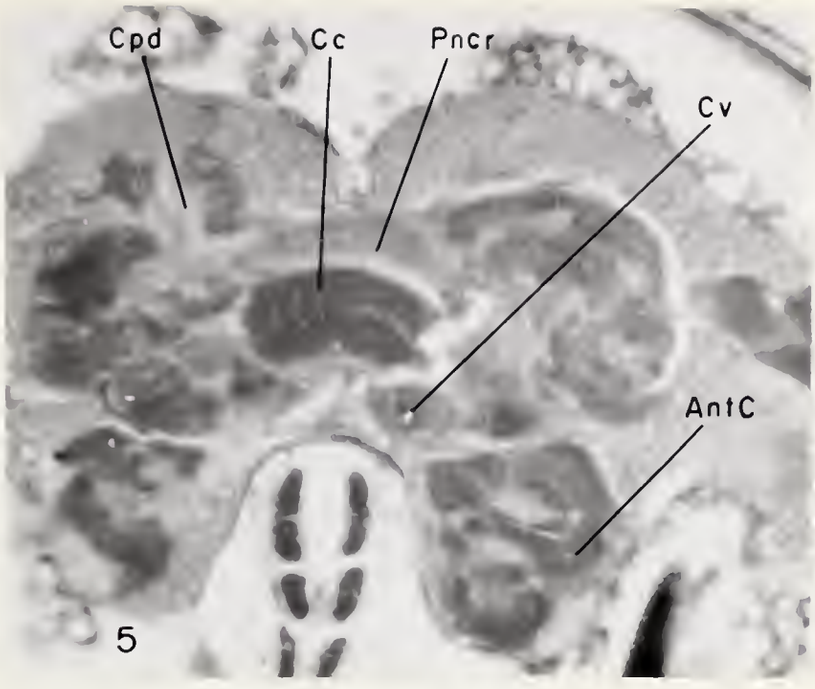
ThGlNv
Oes
Tra
ThGl
4
SalGl

PLATE II.

Fig. 5. Transverse section through brain of fifth instar A. lineolatus nymph. Masson-Bouin; AF; approx. X 230. AntC, antennal centre of deutocerebrum; Cc, corpus centrale; Cpd, corpus pedunculatum; Cv, corpus ventrale; Pncr, pons cerebralis.

Fig. 6. Diagram showing relationship of neuroglandular elements to central nervous system in A. lineolatus adult, dorso-lateral view. CAl, corpus allatum; CPar, corpus paracardiacum; LNsc, neurosecretory cells of pars intercerebralis lateralis; MNsc, neurosecretory cells of pars intercerebralis medialis; NCParExt, nervus corporis paracardiaco externa; NCParInt, nervi corporis paracardiaci interna; Phy, pharynx; 1 Br, protocerebrum.

Plate II.


Cpd
Cc
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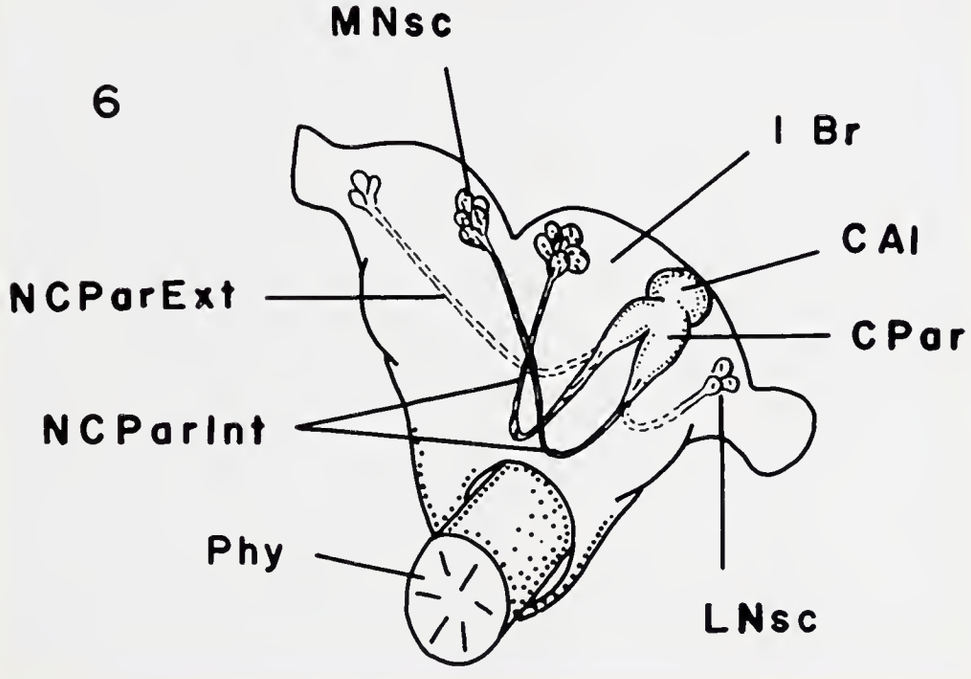
5


6
MNsc
I Br
CAl
NCParExt
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NCParInt
Phy
LNsc

PLATE III.

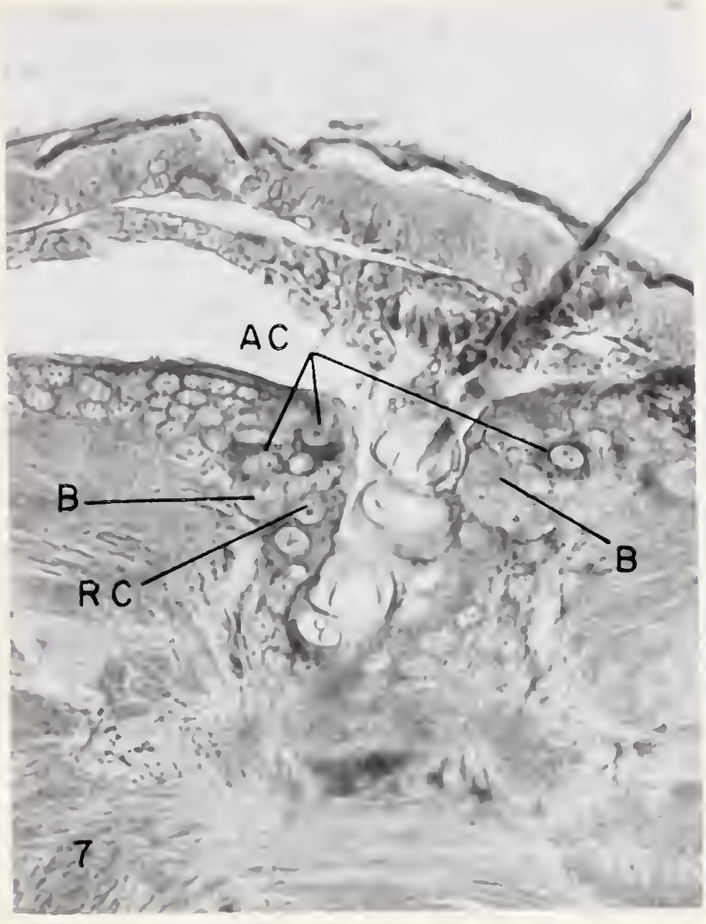
Fig. 7. Frontal section through pars intercerebralis medialis of three-day old fifth instar A. linsolatus nymph, showing types of neurosecretory cells and stages in their secretory activity. Masson-Bouin; AF; approx. X 475. AC, actively secreting Type A cell; B, Type B cell; RC, resting Type A cell.

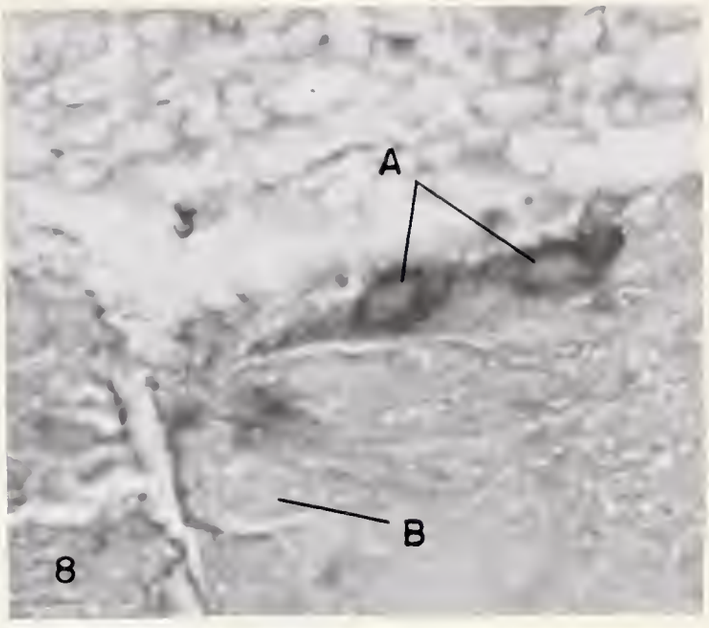
Fig. 8. Frontal section through pars intercerebralis medialis of ten-day old A. lineolatus adult female, showing Type A and B neurosecretory cells and axon transport in A cells. Masson-Bouin; AF; approx. X 900. A, Type A cell; B, Type B cell.

Fig. 9. Frontal section through protocerebrum of three-day old second instar A. lineolatus nymph, showing Type A and B neurosecretory cells. Masson-Bouin; AF; approx. X 400. A, Type A cells; B, Type B cell.

Fig. 10. Frontal section through protocerebrum of three-day old first instar A. lineolatus nymph, showing axon transport of neurosecretory colloid. Masson-Bouin; AF; approx. X 500. A, Type A cells.

Plate III.





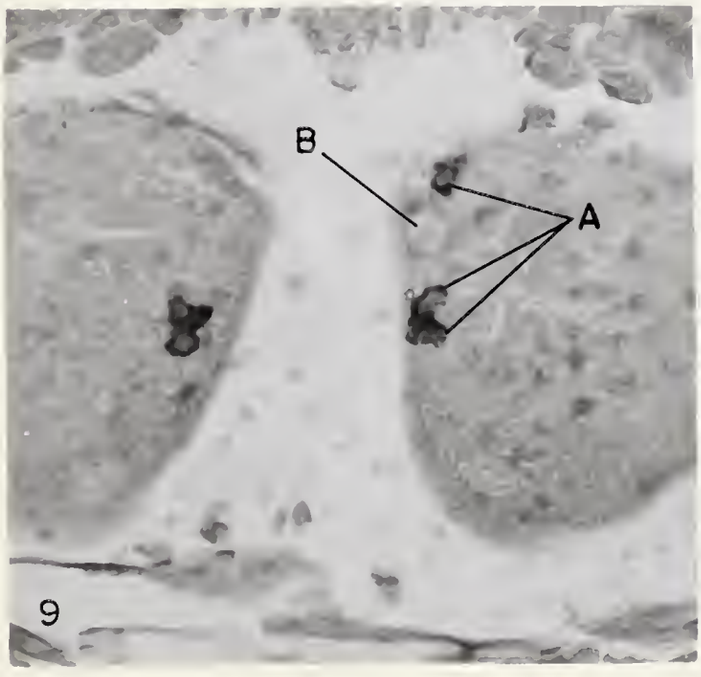


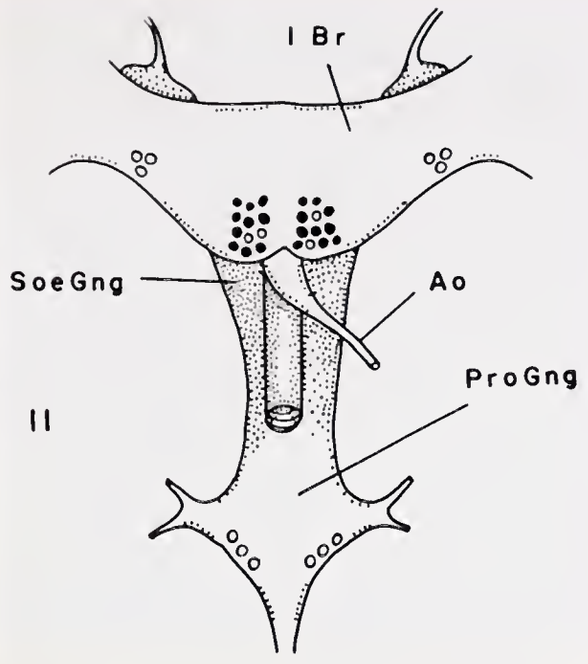
Plate IV.

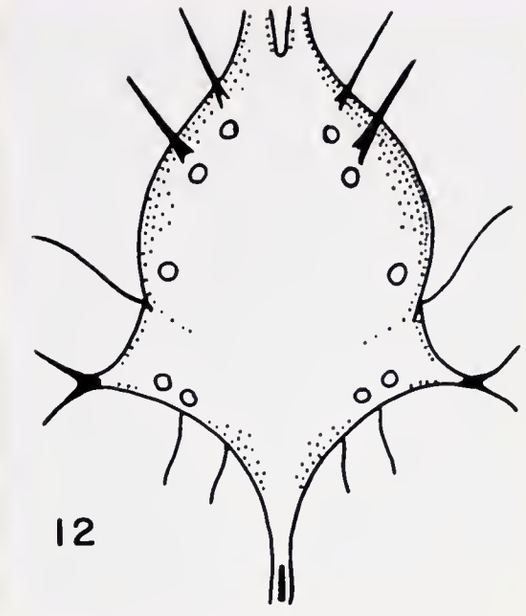
Fig. 11. Diagram showing distribution of neurosecretory cells in the nerve ring of adult *A. lineolatus*, dorsal view. Black dots represent the Type A cells; circles represent the Type B cells. *Ao*, aorta; *ProGng*, prothoracic ganglion; *SoeGng*, suboesophageal ganglion; *l Br*, protocerebrum.

Fig. 12. Diagram showing distribution of Type B cells in thoraco-abdominal ganglionic centre of adult *A. lineolatus*, dorsal view. The six anterior cells are located dorsally, the four posterior cells ventrally, in the ganglion.

Fig. 13. Transverse section through brain of five-day old adult *A. lineolatus* female, showing Type B cells in pars intercerebralis lateralis. Masson-Bouin; CAHP; approx. X 500. *B*, Type B cells; *Cpd*, stalk of corpus pedunculatum.

plate IV.





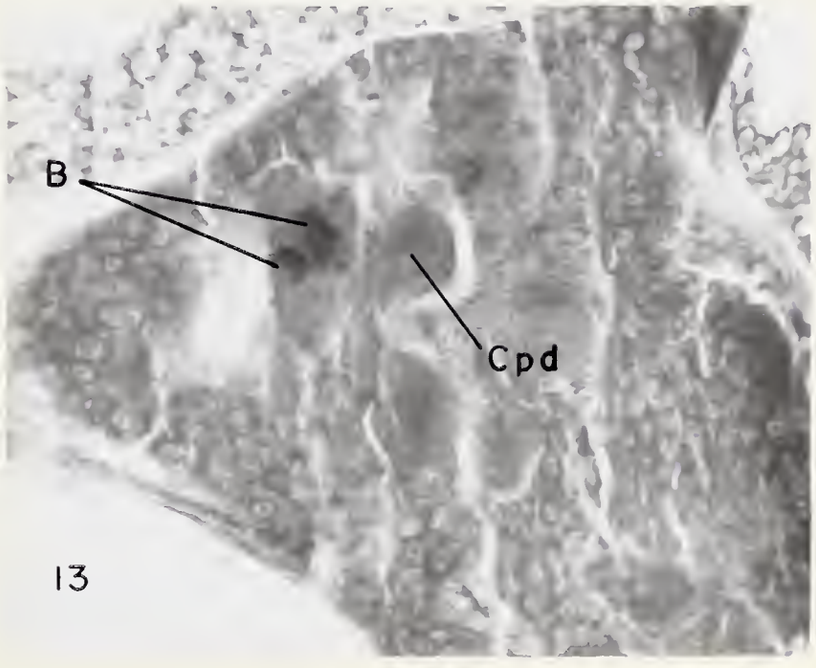


PLATE V.

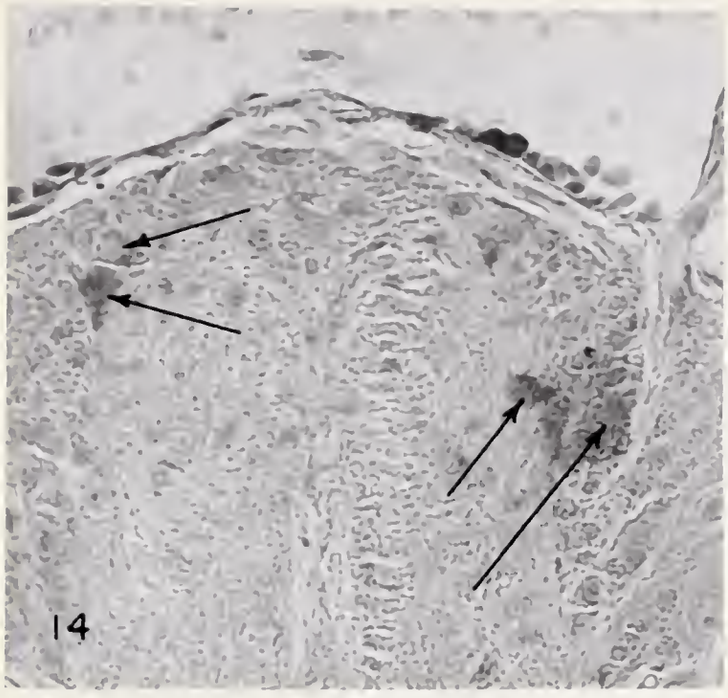
Fig. 14. Frontal section through ventral portion of thoraco-abdominal ganglionic centre of eight-day old adult A. lineolatus female, showing four Type B cells (arrows). Masson-Bouin; CAHP; approx. X 500.

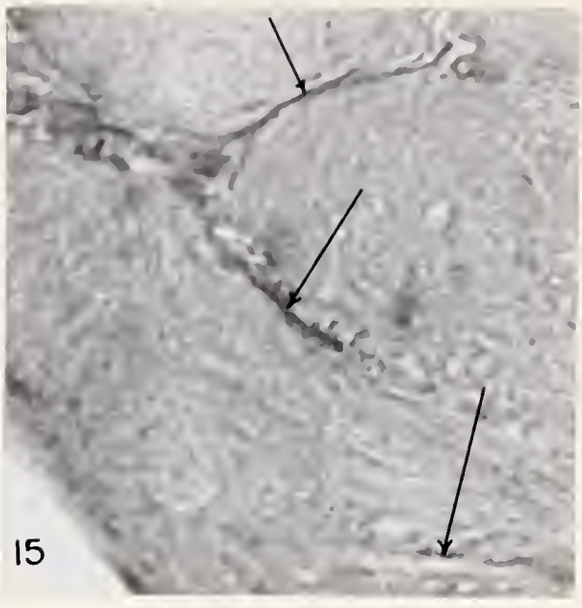
Fig. 15. Frontal section through brain of fourth instar A. lineolatus nymph, showing purple-staining material in neuropile of tritocerebrum (arrows). Masson-Bouin; AF; approx. X 825.

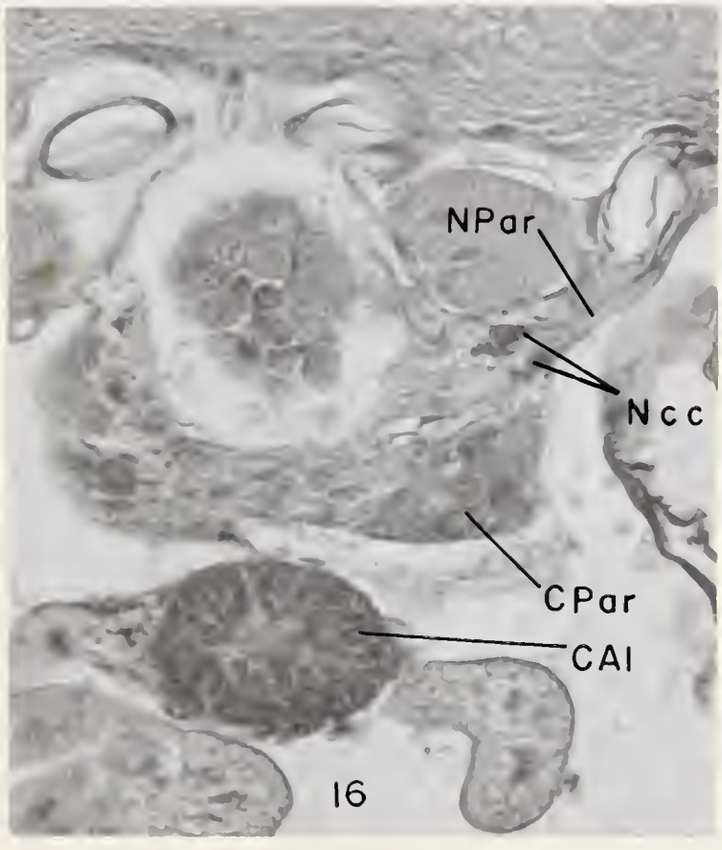
Fig. 16. Frontal section through retrocerebral glands of four-day old fourth instar A. lineolatus nymph. Masson-Bouin; AF; approx. X 575. CAl, corpus allatum; CPar, corpus paracardiacum; NPar, nervus corporis paracardiaco entering anterior portion of corpus paracardiacum; Ncc, neurosecretory colloid.

Fig. 17. Frontal section through corpora paracardiaca of three-day old fifth instar A. lineolatus nymph, showing secretory cells in the gland (arrows) and accumulated neurosecretory colloid (Ncc). Masson-Bouin; AF; approx. X 475. CAl, corpus allatum; Ncc, neurosecretory colloid; NPar, nervus corporis paracardiaco.

Plate V.







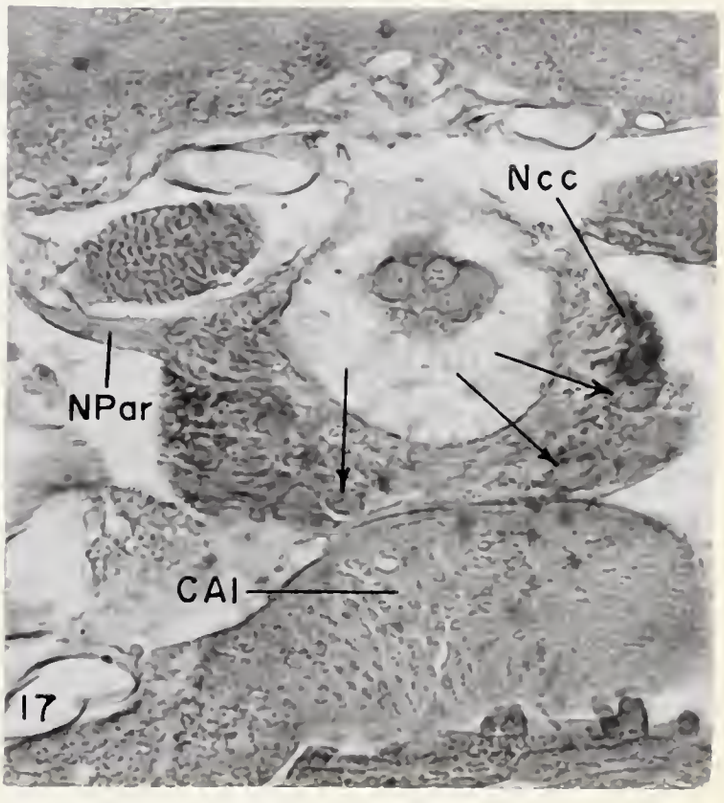


PLATE VI.

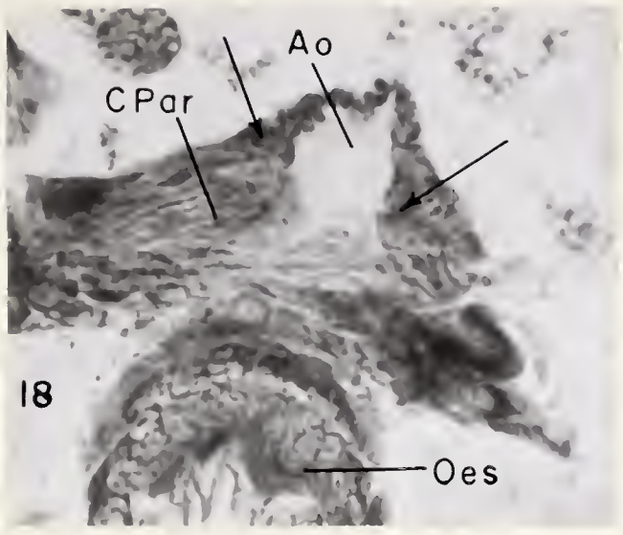
Fig. 18. Transverse section through corpora paracardiaca of three-day old third instar A. lineolatus nymph, showing accumulation of neurosecretory colloid in wall of aorta (arrows). Masson-Bouin; AF; approx. X 500. Ac, aorta; CPar, corpus paracardiacum; Oes, oesophagus.

Fig. 19. Diagram of corpus allatum of four-day old fourth instar A. lineolatus nymph, showing types of cells, dorsal view. Acidophil granules are present in the secretory cells. A few intercellular vacuoles are present. AlN, allatic nerve; IV, intercellular vacuole; Sc, secretory cell; Uc, undifferentiated cell. The corpora paracardiaca are not shown.

Fig. 20. Transverse section through corpus allatum of ten-day old adult A. lineolatus female. The corpus allatum has increased markedly in size. Arrows denote secretory cells. Masson-Bouin; AF; approx. X 300. CAl, corpus allatum; Ncc, neurosecretory colloid; l Br, protocerebrum.

Fig. 21. Section through thoracic gland of four-day old fifth instar A. lineolatus nymph. Masson-Bouin; CAHP; approx. X 625. Mus, muscle; ThGl, thoracic gland.

plate VI.



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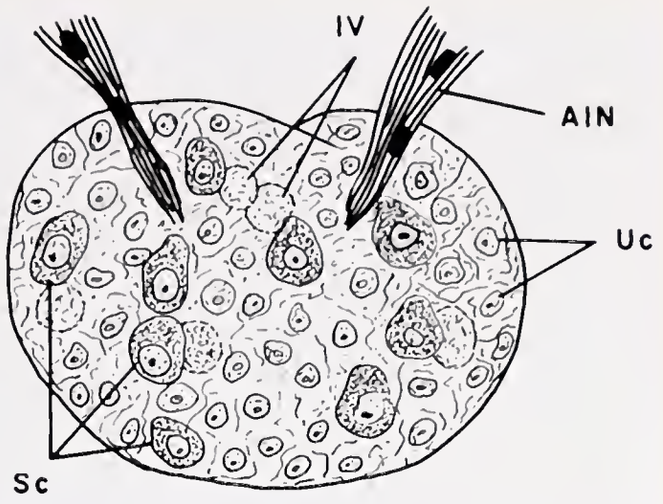

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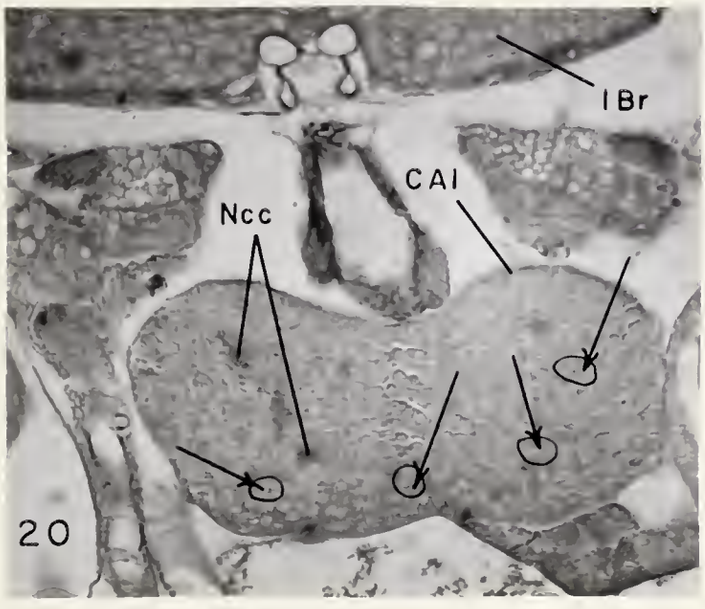

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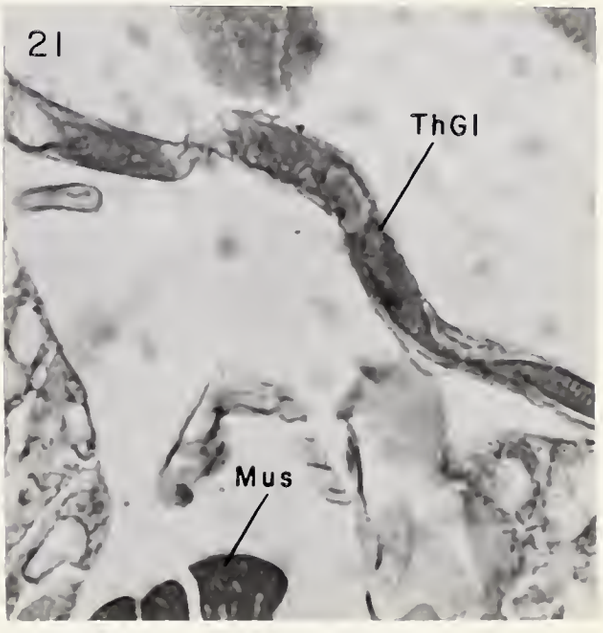

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